Spring 2016

CELLULAR CODING PROPERTIES OF GOAL DIRECTED BEHAVIOR IN THE MEDIODORSAL AND INTRALAMINAR NUCLEUS OF THE RAT: COMPARISONS TO PREFRONTAL CORTEX

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DISSERTATION

Submitted to the University of New Hampshire
In Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
In
Psychology

May 2016

DISSERTATION COMMITTEE PAGE

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On September 11, 2015

Original approval signatures are on file with the University of New Hampshire Graduate School.

DEDICATION

I would like to dedicate this work to my family who have helped me make it through this endeavor.

My children, Kayla and Alex, have supported me every step of the way. Never complaining about the endless nights of takeout while I spent nights in the library and weekends in the lab. I will never forget their words of encouragement that always came when I needed them the most.

My wife and best friend, Kat, I would have never made it without you. You pushed me when I needed pushed and made sure everything else in our lives ran smoothly so I could focus and dedicate my time to completing my dissertation.

My parents, Norman and Frances Miller, who have provided me with a lifetime of support. My mother has always believed in me even when I did not deserve it. My father never wavered in his belief that a was destined for something great even when I did everything to prove to him otherwise. Thank you for believing in me even when I did not believe in myself.

I love you all and I am blessed to have each of you in my life.

ACKNOWLEDGEMENTS

I feel like it has taken an army of support to successfully complete my dissertation. I would like to first thank my co-Advisors: Dr. Robert Mair and Dr. Brett Gibson and committee members, Dr. Robert Drugan, Dr. John Robinson, and Dr. Peter McLaughlin.

Dr. Robert Mair – I can never thank you enough for support you have provided me throughout the past five years. I still believe everything in life happens for a reason. You have helped me grow as a researcher by allowing me to challenge everything, loudly at times. You pushed me to do better and be better every day. "God hates a coward" – *Robert Mair, 2012* I will forever be grateful that you agreed to accept me into your lab.

Dr. Brett Gibson – A simple thank you just does not seem to be enough. You have supported and encouraged me to grow as a researcher and as a professional over the past five years. "Sometimes you get more flies with honey, you can't always bully your way through every situation" – *Brett Gibson, 2012* Thank you for being there to offer advice whenever I lost my way trying to navigate through this process and for pushing me when I needed reminding of why I was here. If I am half the mentor to my future students as you have been to me then I will feel very successful.

Dr. Robert Drugan – Thank you for your constant support over the past five years. You have been an integral part of my success in this program from my master's degree to practicum advisor to completing my comprehensive exams. You have always provided feedback that helped me improve my writing as well as a perspective to look at things with a wider lens. I can

only hope to have the level of enthusiasm and passion for research and teaching that you have when I reach the same point in my career.

Dr. John Robinson – Thank you for taking time to encourage me at every SFN since I first met you in 2009 when I was Pete's undergraduate student. You never hesitated to share your experiences as a graduate student and a mentor that helped me put my own situation into perspective. Plus, it didn't hurt to get advice from someone who had successfully navigated through not only the same program but also with the same advisor.

Dr. Peter McLaughlin – I would not have made it here without your support. I entered your research lab as a sophomore uncertain what I wanted to do in graduate school. "I find what you do fascinating but I don't think it's what I want to do." – *Rikki Miller, 2008* You helped me build a competitive resume by supporting and encouraging me to present our work at a variety of conferences. You helped me prepare for graduate school interviews and provided me with very valuable advice. "Just don't look at their belt" – *Peter McLaughlin, 2010* Most importantly when things in graduate school became difficult you were there to listen and provide me with the encouragement I needed to push forward. Thank you for being a wonderful mentor and friend.

To my fellow graduate students, **Dr. Kristen Onos, Dr. Benjamin Wormwood, and Miranda Francoeur**. Thank you for all your support and for teaching me how much more we can accomplish when we work as team. **Kristen and Ben** thank you for not telling Bob no when he asked if I could join the lab. I am truly fortunate to have had the opportunity to work with all three of you.

Team Thalamus – I would never have completed this work without the dedicated group of undergraduates that helped me in the lab. **Amanda Craig** – you were the first student I

mentored and some days I think you taught me just as much if not more than I taught you. Thank you for sacrificing numerous weekends and hundreds of hours sleep. **Colin Bates** – thank you for all your help building implants and encouraging me never to give up, even after spending days building an implant that didn't work. **Emily Ryder** – thank you for your dedication and commitment to completing the data analysis you were working on before graduating. **Beth Smedley** – you were the first undergraduate to do more than just run the data. You strived to understand what everything meant and how it fit together. I have no doubt that you will be a very successful researcher one day. **Cristy Theriault** – for the record I had to force my computer to stop auto correcting your name. It has been a pleasure to work with you for the past three years. Always remember I wouldn't pick on you if I didn't like you. Your contributions to the project have been innumerable and I cannot begin to thank you for your willingness to reanalyze data files and wave forms time and time again as our knowledge and understanding grew. I knew that I could always count on you to have things done when I needed them. **Erica Brasley** – thank you for all your hard work it was a pleasure to work with you and help oversee your McNair project. **Dan Minukhin** – thank you for giving up a large portion of your summer to work on this project.

Team Tetrode – Thank you to the army of undergraduate students that have worked in the lab throughout this project: Sharon Stevens, Liz Hebert, Andrew Blake, Lydia Manzo, Cora Lehet, Dylan Chase, Ted Kazan, Hayley Robertson, Adam Aasen, Joe McKee, and other members of the Mair/Gibson lab.

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ABSTRACT

CELLULAR CODING PROPERTIES OF GOAL DIRECTED BEHAVIOR IN THE MEDIODORSAL AND INTRALAMINAR NUCLEUS OF THE RAT: COMPARISONS TO PREFRONTAL CORTEX

By

Rikki L.A. Miller

University of New Hampshire, May 2016

The mediodorsal (MD) and rostral intralaminar (IL) nuclei of central thalamus interact with prefrontal cortex (PFC) through multiple pathways to control goal directed behavior. The initial purpose of this dissertation was to characterize cellular coding properties of these nuclei in central thalamus using electrophysiological measures in awake, behaving rats performing a dynamic delayed non-match to position (DNMTP) task. Two major aims were developed. The first of these was based on the strong reciprocal connections between central thalamus and PFC. Therefore, the current data was compared to data previously collected in prefrontal cortex (Onos et al., 2015). The second was that despite to evaluate the coding properties of MD and IL to elucidate the differences and similarities of cell types found in each thalamic nuclei. Tetrode arrays were implanted and advanced incrementally through MD and IL. A total of 1335 thalamic cells were recorded, 385 (29%) of which were behaviorally correlated (144 in MD and 241 in IL). In general, behaviorally correlated cells fell into one of three broad categories used to define goal-directed behavior (Action, Outcomes, and Action/Outcomes). As expected there was a great deal of overlap in cell types found in central thalamus and PFC as well as many differences. Results suggest that cells within MD are primarily responsible for coding of reinforcement and movement related activity. In addition, cells recorded from IL appear to code for more complex aspects of the task.

INTRODUCTION

The mediodorsal (MD) and rostral intralaminar (IL) nuclei of central thalamus interact with prefrontal cortex (PFC) through multiple pathways to control goal directed behavior. MD and IL are both reciprocally connected to PFC and also receive indirect inhibitory input from PFC through cortico-striato-pallidal pathways (Groenewegen, 1988; Groenewegen et al., 1990; 1999; Hoover & Vertes, 2007; Xiao, Zikopoulos, & Barbas, 2009). In addition, IL provides the main thalamic input to striatum (Groenewegen & Berendse, 1994; Berendse & Groenewegen, 1991; Krout, Belzer, & Loewy 2002; Van der Werf, Witter, & Groenewegen, 2002; Vertes, Hoover, & Rodriguez, 2012; Figure 1). Traditionally, these nuclei have been referred to as specific (MD) and non-specific (IL) due to the precise and diffuse termination fields in PFC of the rat (Groenewegen & Berendse, 1994; Krout et al., 2002; Voorn et al., 2004; Vertes et al., 2012). The projections from MD terminate primarily in cortical layer III and receive afferent connections from layer V (Groenewegen, 1988; Groenewegen et al., 1990; 1999; Hover & Vertes, 2007; Xiao et al., 2009). Berendse and Groenewegen (1994) found that projections to PFC from IL also terminated in very specific fields located primarily in cortical layer I and receive projections back from layer VI, suggesting that IL may not be as 'non-specific' as once believed (Berendse & Groenewegen, 1994). The connections from these nuclei have been hypothesized to be involved in higher order cognitive activity involved in goal directed behavior (Saalmann, 2014).

Studies conducted by Sherman and Guillery (1998; 2011; 2012) have investigated the thalamo-cortical pathways further and identified that glutamatergic circuits can be divided into

two classes based on functional and anatomical properties. Class one (driver) inputs activate only ionotropic glutamate receptors and produce larger EPSPs compared to class two (modulatory) inputs. The main distinction is that class two inputs also activate metabotropic glutamate receptors, which can produce excitatory or inhibitory actions. The driver inputs produce an excitatory pathway, thus 'driving' the activity in areas of termination, whereas modulatory inputs produce excitatory and/or inhibitory actions, thus 'modulating' the activity in areas of termination. Additional work has provided a more detailed understanding of these pathways in which thalamus can be divided into first order and higher order relays (Sherman, 2012). First order nuclei receive class one input from a subcortical source and higher order nuclei receive input from cortex. The role of higher order nuclei is to relay information between cortical areas. This evidence supports the hypothesis that MD provides the main driver input to PFC from thalamus and IL provides the main modulatory input. MD and IL have also been identified as higher order nuclei as they receive input from cortex (Groenewegen et al., 1988; Sherman 2012; Saalmann, 2014).

It was the aim of this dissertation to further investigate the role of the three sub-regions of MD: medial MD (MDm), central MD (MDc), lateral MD (MDl), and three nuclei that make up the rostral IL: the central lateral (CL), paracentral (PC), and central median (CM) nuclei in goal directed behavior. These nuclei are of interest due to the specific anatomical connections to cortical regions known to be involved in goal directed behavior (Groenewegen, 1988; Berendse & Groenewegen, 1991; Krout, et al., 2002; Vertes, et al., 2012; Voorn et al., 2004; Bailey & Mair, 2005; 2007; Chudasama, 2011; Chudasama and Robbins, 2006) as well as inputs from brainstem regions involved in arousal and attention.

The current hypothesis is that cortical activation associated with arousal and attention stems from the reticular formation, routed through IL because of its strong afferents from the reticular formation (Groenewegen & Berendse, 1994; Berendse & Groenewegen, 1991; Krout, et al., 2002; Van der Werf, et al., 2002; Vertes, et al., 2012;Voorn et al., 2004). If the IL were a relay for arousal and attentional information then stimulation of this should result in increases of both systems. A study conducted by Mair and Hembrook (2008) supports this hypothesis. Stimulation of the IL in rats was found to improve performance during a delayed match to position (DMP) task but only at event specific times. Low levels of stimulation given prior to each phase of the DMP task (i.e. start, sample, delay, and choice) were found to improve accuracy. Further analysis of each phase showed enhancement of performance was only found with stimulation prior to phases that are most taxing on executive function, delay and choice. The delay and choice phases of this task require information to be held in working memory or retrieved from long-term memory depending on the length of the delay (Mair & Hembrook, 2008; Chudasama, 2011). Anatomical studies have shown that MD also receives afferents from brainstem areas; however, these afferents have more diffuse distribution (Groenewegen, 1988). The effects of these various brainstem afferents may depend on the precise regions of cortex that MD and IL project to. It is therefore important to evaluate the specific point-to-point connections between thalamus and PFC as well as their individual influences on behavior.

Lesion studies have been used to evaluate the influence of specific brain regions on observable behavior. Lesions to PFC, MD, and IL have been shown to impair goal directed behaviors, including delayed conditional discriminations and other tasks used to assess working memory (Mitchell & Charkraborty, 2014; Baily & Mair, 2005; Chudasama, 2011). Bailey and Mair (2005) found that thalamic lesions impaired performance on PFC dependent tasks (delayed

match with retractable levers; DMRL) but had no effect on hippocampal dependent (varying choice delayed non-match) tasks. They also investigated the effect of lesion size, comparing large lesions encompassing MD and IL to discrete lesions of MD and IL individually using DMRL. Differential effects of discrete lesions and large lesions were found, both large lesions and discrete IL lesions impaired performance in a delay independent manner. In contrast, discrete lesions to the MD impaired performance in a delay dependent manner (i.e. performance was worst at the longest delay) (Bailey & Mair, 2005). The results suggest that MD is required to hold information 'online' during a delay to guide behavioral actions, whereas IL is thought to play a role in activating PFC and anatomically connected areas of striatum, thus normal PFC function depends on input from IL (Bailey & Mair, 2005). Damage to IL was found to cause a broad disruption of PFC function. Other studies have attributed the deficits caused by IL lesions to a decrease in the flexible use of information, not as a global dysregulation of working memory (Van der Werf et al., 2002). To further understand and investigate the differential involvement of MD and IL it is important to consider the similarities and differences in anatomical connections to PFC.

Specific cortical projections from the MDm terminate in prelimbic (PL), infralimbic (ILc), medial and ventrolateral orbital frontal (MO; VLO), and ventral anterior cingulate (ACv) (Groenewegen, 1988; Groenewegen et al., 1990; 1999; Hover & Vertes, 2007; Xiao, Zikopoulos, & Barbas, 2009). In non-human primates lesions to MDmc, which is comparable to a portion of MDm in rodents, were found to impact learning of new information but had no effect on retention of old information. In addition these lesions were found to impair reward devaluation learning (Mitchell & Charkraborty, 2014), suggesting that MDm may play a role in learning of new outcome expectancies.

MDc has projections to lateral orbital (LO) and ventral agranular insular cotices (AIv). MDl sends projections to ventral orbital (VO), dorsal anterior cingulate (ACd), medial precentral cortex (FR2) and dorsal agranular insular cortex (AId) (Groenewegen, 1988; Groenewegen et al., 1990; 1999; Hover & Vertes, 2007; Xiao, Zikopoulos, & Barbas, 2009). Less is known about the behavioral effects of specific lesions to MDc and MDl.

Lesions to the interconnected regions of PFC have been shown to impair performance on tasks used to evaluate working memory, attention, and decision-making in primates and rats (Chudasama, 2011; Uylings, Groenewegen, & Kolb, 2003; Table 1). More specifically, lesions to PL impair performance on tasks of working memory (delayed response) and attention (five-choice task), this suggests that PL, like MD, is important for holding information 'online' during a delay to direct and guide future actions (Chudasama, et al., 2003; Chudasama, 2011; Uylings, Groenewegen & Kolb, 2003). In addition, the increases in perseverative responses during the five-choice task are indicative of an inability to disengage from repeating a correct response (Chudasama, 2011) and could reflect a deficit in updating outcome expectancies. Lesions to ILc have no effect on perseverative responding but greatly increase the number of premature responses suggesting that ILc plays a role in behavioral inhibition (Chudasama, et al., 2003; Chudasama, 2011). Orbital frontal cortex (OFC) is sensitive to changes in task demands and reward contingencies (Schoenbaum, Nugent, Saddoris, & Setlow, 2002; Chudasama, 2011; Uylings, Groenewegen, & Kolb, 2003). Rats and monkeys with lesions to the OFC have shown impaired performance of reversal learning. Lesions also produce impairment of go/no-go task performance, specifically an inability to inhibit responding in the presence of the no-go stimulus (Chudasama et al., 2003; Chudasama, 2011; Uylings, Groenewegen, & Kolb, 2003). Lesions to AI have been shown to impair working memory for food reward value in a delay dependent

manner (Ragozzino & Kesner, 1998; Uylings, Groenewegen, & Kolb, 2003). All of these deficits point to a lack of cognitive flexibility, when already established rewards expectancies must be updated and behavior adjusted accordingly.

Nuclei of the rostral IL have reciprocal projection to PFC and non-reciprocal projections to striatum (Groenewegen & Berendse, 1994; Berendse & Groenewegen, 1991; Krout, et al., 2002; Van der Werf, et al., 2002; Vertes, et al., 2012; Voorn et al., 2004). Voorn and colleagues (2004) identified that PFC also has non-reciprocal projections to striatum. The non-reciprocal projection from IL and PFC to striatum are organized in such a way that the regions of IL and PFC with reciprocal projections terminating in similar regions of striatum. CL projects to sensorimotor cortex (SMC) and both CL and SMC have non-reciprocal projections to the same region of dorsal striatum (Voorn, et al, 2004). PC shows a similar pattern of connectivity to the dorsal anterior cingulate (ACd), with reciprocal connections between them and non-reciprocal projections to dorsal striatum (Groenewegen et al., 1994; Voorn, et al., 2004). CM has reciprocal connections to dorsal PL and dorsal lateral orbital frontal (DLO) and both regions have non-reciprocal projections to medial dorsal striatum (Groenewegen et al., 1994; Voorn, et al., 2004 Vertes et al., 2012). These regions of cortex have been investigated for their role in reward-guided decision-making with tasks such as delayed discounting (Table 2). Rats with OFC lesions will choose small immediate/certain rewards over large delayed/uncertain rewards (Chudasama, 2011). Rats with lesions to AC however did not base decisions on the cost of the delay but on the cost of physical effort to obtain the reward (Chudasama, 2011), suggesting that OFC and AC process different aspects of reward-guided decision-making. In contrast lesions to motor areas (SMA & FR2) appear to have no effect on accuracy, but do impair the speed of responding (Chudasama, 2011; Uylings, Groenewegen, & Kolb, 2003) and initiation of learned

action sequences (Bailey & Mair, 2007). Based on this information it is reasonable to hypothesize that the behavioral function of each sub-nucleus of both MD and IL will be similar to the behavioral function of the interconnected region of PFC.

While lesions are useful for demonstrating the necessity of brain regions for behavioral functions, they are of limited utility for understanding the precise roles of local brain regions in complex functions. Electrophysiological studies of single neuron activity provide a powerful tool for understanding brain function at a cellular level and thus to examine functions of local areas that are too small to produce measurable deficits in lesion studies. Early electrophysiology studies described delay-related increases in activity observed in prefrontal neurons in primates and rodents performing delayed response tasks and it was hypothesized that these responses represent an active working memory store essential for prefrontal function (Baeg, et al., 2003; Chang, et al., 2003; Funahashi, 2006). More recent studies have emphasized prefrontal responses related to the planning and execution of actions as well as anticipation and monitoring of action outcomes (Tanji and Hoshi, 2008; Euston and McNaughton, 2006; Insel and Barnes, 2014; Horst and Laubach, 2012; Matsumoto, et al., 2003). Less is known about the activity of central thalamic neurons in these tasks.

Electrophysiological studies with non-human primates described delay-related activity found in MD and IL that is comparable to PFC (Funahashi, Inoue, & Kubota, 1993; 1997; Wyder, Massoglia, & Stanford, 2003; 2004) as well as activity related to attention and arousal (Matsumoto et al., 2001; Minamimoto and Kimura, 2002). One study using non-human primates provided support for the cortical-striatal-thalamic connection, by which the IL is thought to relay behaviorally significant information to the striatum. Matsumoto and colleagues (2000) performed single cell recording in the IL and striatum of non-human primates. They found

increases in patterns of firing rates hypothesized to be involved in relaying behaviorally significant information to the striatum (Matsumoto, Minamimoto, Greybiel, & Kimura, 2000). Neurons in the IL showed minimal activation when presented with several novel sensory stimuli (e.g. click, beep, or LED). The click was paired with reinforcement and an increase in neuronal activity for the click and reinforcement was found, firing rates became identical for the click and for reinforcement. The activity was to be expected based on standard classical conditioning theory, however, in subsequent trials when no reinforcement was provided neuronal activity for all three stimuli (click, beep, and LED) was increased, mimicking the pattern of activity found for the click/reinforcement trials. The beep and LED were never paired with reinforcement, yet the neuronal response in the IL to these stimuli showed generalization to reinforced trials. Responses of tonically active neurons in the striatum did not reflect this generalization, activity was only found for the click/reinforcement trials. Neuronal activity to the sensory stimuli was shown to habituate during a block of 25 trials consisting of just the click stimulus without reinforcement resulting in a decrease in responding. This suggests that once it is determined that information is no longer behaviorally significant, responding decreases. The striatal response to the click can be eliminated by inactivation of IL with muscimol, validating the communication of the relevant (click = reinforcement) behavioral information from the IL to the striatum. These findings suggest that IL input relays information about the appearance, disappearance, and changes of attention demanding behaviorally significant events. Results also support the contribution of thalamo-striatal loop to the function of the basal ganglia related to the selection of forthcoming events (Matsumoto et al., 2000).

Despite new opportunities afforded by current technology, it is difficult to compare findings across studies due to variation in tasks and procedures used for data analysis. There are

exceptions to this that allow for a more thorough evaluation of brain regions believed to be parts of a network or circuit. For example, Chang and colleagues (2003) recorded simultaneously from PFC, dorsal striatum and nucleus accumbens (NAc) of rats preforming a DMTS task. This allowed them to directly compare cellular activity in these regions. The benefit of the DMTS task is that it has very distinct stages that can be identified and changes in cellular responding immediately before and after each action (sample, end of delay, match) can be assessed. They were able to identify significant changes in cellular firing patterns to all three actions. These changes were initially categorized as excitatory, inhibitory, or bi-modal (displaying both excitatory and inhibitory changes) for each action. Delay related activity similar to that reported in non-human primates was found. Further analysis showed cellular coding for location, such as significant changes in firing rates of one neuron in response to sample on lever A versus sample on lever B. In addition to location they found a population of cells that differentially responded to correct responses versus errors. Interestingly, when correct and error trials were evaluated in the NAc during the delay (before the choice), to be correct trials indicated an increase in activity during the delay. This delay-related activity is similar to that found in PFC, which is believed to be involved in holding information 'online' in working memory. The authors inferred involvement of this system in learning and memory processes. However, they also acknowledge that the study is not without its faults. The delay period used is much longer than what is commonly accepted as working memory (20-30 s) as well as inconsistencies in their findings across cellular patterns, especially in regard to correct/error coding that could not be fully explained. A major aspect of this task that was not accounted for or evaluated was the effect of reinforcement on subsequent behavior. Given that PFC and NAc are areas associated with reinforcement this could be a key element missing from previous research. Similarly, Han, et al.

(2013) wanted to compare firing patterns of PFC to MD. To do this they replicated the task (delayed alternation task) and analysis of a previous study of PFC conducted by Baeg and colleagues (2003) they recorded MD neurons in rats. (Jung, et al., 1998; Baeg, et al., 2003). They report that MD neurons do not exhibit elevated activity throughout the delay period like that previously observed in PFC. However, they did observe elevated activity associated with reinforcement, which they interpret as evidence that MD is involved in response reward associations (Han et al., 2013).

It was the goal of this dissertation to characterize response properties of MD and IL using electrophysiological measures in awake, behaving rats. Recent work in our lab has characterized response properties of prefrontal neurons in rats performing a dynamic DNMTP task (Onos et al., 2015). Given the strong interconnections of MD and IL to prefrontal cortex this seemed to be the most reasonable starting point to elucidate the cognitive coding properties of central thalamic neurons. Response properties of neurons found in MD and IL of rats performing a dynamic delayed-nonmatch-to-position (DNMTP) task in an open arena with visible external cues available were analyzed. The dynamic DNMTP task is based on procedures shown to be sensitive to the effects of PFC lesions (Porter, Burk, & Mair, 2000). The task is dynamic in that the start location changed randomly between trials to increase demands on flexible responding. Retractable levers are used to provide precisely timed behavioral events to distinguish responses from movement related activity. The same task and measurements were used previously to characterize cellular coding in PFC, including event related analysis using perievent histograms/rasters and place analysis to asses context coding (Onos et al., 2015). In addition to direct comparisons to PFC activity, cellular activity found in MD and IL was compared to evaluate the role central thalamus in goal directed behavior.

METHODS

Subjects

9 male Long Evans rats were obtained from Harlan Laboratories (Boston, MA) at approximately 3 weeks of age. Animals were housed in pairs for approximately one week and allowed to acclimate to the animal vivarium before separation and were then handled daily. Housing consisted of a 33 x 21 x 33 cm plastic tub cage filled with wood shavings. The vivarium is maintained on a 12:12 h light:dark cycle with lights on at 07:00. All training and testing took place during the light phase. All animals were allowed *ad libitum* access to food and water until they reached a weight of 250 grams, at which point a water restriction schedule began. Animals only received water as reinforcement during training and for 30 minutes at the end of each training/testing day and one h on non-training/testing days. All animals were monitored daily for health concerns. The Animal Resource Office provided any necessary care. Approval of the Institutional Animal Care and Use Committee (IACUC) of the University of New Hampshire was granted for this project (IACUC 110901).

Apparatus

A custom built operant box (60 x 60 x 34) was used to carry out a modified version of the delay non-match to position task (DNMTP). The apparatus is octagonal and made of clear polycarbonate walls with a white painted wood floor. The four corners (N, E, S, W) are identical each containing a retractable lever (6.5 cm from the floor), signal light (2.5 cm in diameter and 4 cm above the lever), and water port (6 cm above the light). Reinforcement (2 pulses equivalent

to 0.1 ml of tap water) was delivered by activation of solenoid valves (LFAA1201518H, The Lee Co., Essex, CT). The top of the apparatus is open to allow the tether to be connected to record activity of awake, behaving rats. The chamber is located in a grounded Faraday cage to minimize external electrical interference (Figure 2). A Dell computer located in an adjoining room is connected to an interface (Med Associates) and uses software developed by MED-PC (Georgia, VT) to control the apparatus. The chamber was spot cleaned between sessions and cleaned at the end of each day with a residue free soap and hot water.

Behavioral Task

The behavioral task is a dynamic variation of the DNMTP. During a given session the start levers were pseudo-randomly selected from two possible locations (NS or EW), such that the possible locations were alternated from one session to the next (i.e. Day 1 NS; Day 2 EW; Day 3 NS; ect.). A response on the start lever caused the lever to retract and the sample lever was randomly selected, either left or right of the start lever. A response on the sample lever caused it to retract and the original start lever (now the delay) to extend. The first response after a 3 s delay caused the delay lever to retract and the levers to the left and right to extend. A response on the lever that was not the sample was recorded as correct, the light above the lever illuminated signally that reinforcement is available (Figure 3). A response on the lever that matches the sample was recorded as an error and no reinforcement was delivered. The levers remained extended until a correct response is made. A response on the correct lever ended the current trial and begin a fixed 5 s inter-trial interval. This pattern continued until the rat completed 60 trials or times out at 60 minutes. Rats were required to perform at 70% criterion

and complete all 60 trials for at least 3 consecutive days before undergoing surgery. Training to criterion takes approximately 4-5 months.

Surgical Procedure

All surgical instruments was sterilized prior to surgery in an autoclave or, if the tools would not fit in the autoclave, by submersion 70% ethanol. Rats were anesthetized using a combination of Ketamine/Xylazine (80/8 mg/kg), injected (intramuscular) using a 1 ml syringe with a 25 gauge stainless steel needle. Once deep anesthesia is achieved, any hair in the surgical field was shaved off and ointment was applied to the eyes to prevent drying. At this point the rat's head was secured with atramatic ear bars into the stereotaxic instrument and the skin in the area of the incision was cleaned with Betadine. An incision was made along the midline of the skull and the skin was retracted to expose the surgical field. The stereotaxic plane was verified by measuring the locations of Bregma and Lambda sutures relative to the interaural line. Stereotaxic coordinates for the implantation of the custom built tetrode (see recording equipment) were measured in mm relative to the interaural line for anterior-posterior (AP) and dorsoventral (DV). The tetrode tips were aimed at +4.8 mm DV and +6.2 mm AP. Target midline location varied as follows implants per location: $\pm$0.4 mm (MDm), $\pm$0.6 mm (MDc), $\pm$1 mm (MDl), and $\pm$1.2 mm (IL; see Histological results for exact placement).

Tetrode and machine screws were sterilized using the same procedures used for surgical instruments. Eight small holes were drilled in the skull around the implantation site so that stainless steel machine screws could be inserted to anchor the tetrode assembly in place. Screws were placed so that they do not reach through the skull to the dura mater and their tops extend above the skull. The skull was then opened using a burbit and the dura removed. The tetrode

was then lowered to the desired coordinates using the stereotaxic instrument. The tetrode was held in place while grip cement was applied around the site and allowed to harden (generally taking about 10 minutes) so that it secured the external portion of the tetrode to the skull and the partially exposed machine screws.

The inner sides of the incision were carefully opposed and the incision was closed, around the tetrode assembly, with sutures. The suture line was irrigated with Betadine and the rat was transferred to a clean cage where it was kept warm and monitored until it recovered from the effects of the anesthesia. Rats were administered Butorphanol via SC injection using a 1 ml syringe with a 25 gauge stainless steel needle following surgery. This was done to alleviate irritation caused by surgery, particularly from the implanted tetrode array, and speed recovery from surgery. All animals were allowed to recover for one week and given *ad libitum* access to food and water.

Recording Equipment

Activity was recorded with a drivable array of four tetrodes custom built and implanted at one of several recording sites. Tetrodes were fabricated by twisting together four 17.8 micron platinum iridium wires, which were then bundled together and threaded down two separate guide cannula. Each cannula contained two tetrodes and was placed bilaterally across midline to record from the same location in both hemispheres simultaneously. The wires were then individually wrapped around 16 pins of a Mill-Max$_{TM}$ connector and encased in cranioplastic cement. The custom-built tetrode assembly contains three screws, which were used to slowly drive the array. Prior to implanting, the tetrode array was tested for proper impedance with

Nano-Z program and equipment (Neuralynx) and, when needed, platinum black coating was applied to the wires to decrease impedance to ≤ 250 mega ohms. The tetrodes were documented so that each tetrode can be analyzed based on the hemisphere it was implanted in.

The Cheetah data acquisition system (Neuralynx) was used to record data simultaneously from the four tetrodes. A low torque slip-ring commutator (Dragonfly Research and Development Inc.) was housed inside the faraday cage above the operant box. A 18 pin Mill-Max$_{TM}$ headstage (Neuralynx) was connect the tetrode assembly to the commutator, which prevented the cable from becoming twisted as the rat moved around the chamber. Recorded raw signals were amplified and sent to the Neuralynx Digital Lynx 4SX to be processed using Cheetah data acquisition software. Thresholds were set manually each day based on the signal being collected.

A super port (DIG-726-TTL card) connected to the interface (Med Associates) sent TTL pulses to the Digital Lynx 4SX. These TTL pulses record time stamps to specific events within the task (i.e. start, sample, delay, and choice). The TTL pulses were used to correlate neural activity at the time of each specific event (Table 3). The Neuralynx system also allowed for video tracking of the daily sessions. Two LEDs, red and blue, attached to the headstage tether allowed for moment-to-moment tracking of the rat's position as well as head direction.

Microdrive Procedure

At the end of each session rats were wrapped in a towel and hand held while a jeweler's screwdriver was used to manually turn the tetrode assembly screws one sixteenth of one turn, equal to 0.028 mm, this drove the tetrode array into more ventral regions of thalamus.

Histological Analysis

After recording sessions are completed the end of the electrode track was marked for better visualization. A 100 μv current was passed along two of the four tetrode bundles (one in each hemisphere) for 30 s and allowed three days for gliosis to occur before being sacrificed. Rats were euthanized (100 mg/kg ketamine, 10 mg/kg xylazine) and underwent transcardiac perfusion of 0.9% physiological saline followed by 4% (v/v) neutral buffered formalin. Brains were then extracted from the skull and immersed in 30% sucrose in 4% neutral phosphate buffer until they sunk. Tissue was sectioned frozen in the coronal plane at 50 μm and stained with thionin Nissl stain in order to verify the tetrode placement and electrode path as well as assess any tissue damage.

Data Analysis

Data consisted of a continuous digital recording for each daily session, TTL pulses marking behavioral events, and HD video tracking. Data was analyzed by tetrode, which contained the data for four individual microwires. Data was first cluster cut offline to identify waveforms. SpikeSort3D software (Neuralynx) was used to run the auto-clustering program KlustaKwik that compares changes in amplitude across all individual recorded spikes on a variety of measures (i.e. peak height, valley, and width) to group together similar activity from each tetrode. Once KlustaKwik grouped the spikes into clusters each cluster was further evaluated to identify single cells. The preset criteria for identifying isolated cells used in analysis of previously collected PFC data will be used. The criteria were as follows: cells were distinct waveforms recorded from different microwires in a tetrode, a well-defined cluster in the 3D plot, an interspike

histogram above 1 ms peaking above 10 ms, signal to noise ratio of 1.5:1, hyperpolarization that was asymmetrical with depolarization and an L ratio <1 (Onos et al., 2015; Figure 4). Once a waveform was identified as an isolated cell NeuroExplorer software (Madison, AL) was used to create rasters and peri-event time histograms (PETH) relative to TTL pulses of behavioral events. Cells were identified as behaviorally correlated only if they exceed the 99% confidence interval for multiple time bins on the PETH and were associated with consistent changes in activity in the rasters. Behaviorally correlated cells were compared to patterns of activity (PETH and rasters) found in PFC. NeuroExplorer software was used to create placemaps of the available video tracking data to evaluate the spatial distribution of activity associated with behaviorally correlated cells.

RESULTS

Histological Analysis

Tissue from seven rats was examined; two rats are currently still being recorded. The histology from the rats was used to identify the tetrode placement (Table 4). Five rats were found to be in MD, three unilateral (single cannula) and two bi-lateral (dual cannula), four tracks in these animals were identified as driving through MD into IL. In these five rats recordings were collected from MDm, MDc, and MDl (Figure 5, 6, 7, 8, 9, & 10). Implants in two rats were found to be in IL, both of these were unilateral (single cannula). Including the four rat with MD/IL recordings the histology shows that cells were recorded from all three nuclei of IL: CL, PC, and CM (Figure 11, 12 & 13). The target location of the two animals currently still being recorded is within MD aiming for MDc and MDm (Table 4). The cell recorded to date from one of these animals has been included in the cell counts. The current data fits with the trend found in MD recordings and there is no reason to suspect that our implant is not close to the target location.

Electrophysiological Analysis and Comparisons

Across all eight animals in central thalamus a total of 1335 neurons were recorded, 385 (29%) were behaviorally correlated. Of the 385 behaviorally correlated neurons 346 (90%) fell into one of three broad categories used to define goal-directed behavior (Action, Outcomes, and Action/Outcomes). 570 (43%) of these neurons were recorded in MD (Figure 10), 144 (25%) were behaviorally correlated. Of the 144 correlated neurons 128 (89%) fell into the previously mentioned categories. The remaining 753 (56%) neurons were recorded in IL (Figure 13), 241

(32%) of the neurons were found to be behaviorally correlated and 218 (90%) fit into the same categories (Table 5).

Each of the broad categories was broken down further into specific cellular activity that appears to be driven by or focused around behavioral events within the task. The category Action contained 4 groups of cell types: movement, lever press, delay, and preparatory. The first group, movement related activity, cells in this group respond at significantly higher levels when the animal is moving about the arena. Cell types classified as movement related make up 52% of all correlated cells found in central thalamus (Table 6). There are four movement related cell types found in MD and IL as well as PFC (Onos et al, 2015).

Movement 1 (Figure 14 & 15)

Characterized by an increase in firing rate 1-2 s before the lever press with an abrupt decrease in firing rate at the time of the lever press lasting from 0.5-1 s at start and delay responses and 1.5-2 s at sample and correct responses. This activity was deemed as movement related because the decrease in firing rate corresponds to time in the task when the animal is stationary thus the longer decrease found at sample and reinforcement because the animal remains at the lever location longer to receive reinforcement. Movement 1 (M1) cells were by far the most abundant of all cell types found. They make up 26% (N=91) of all classified cells in central thalamus (Table 5). Although M1 cells are found in both MD and IL they make up a greater percentage of the classified cells found in MD (40%) compared to IL (17%; Table 6). M1 was previously identified in PFC and was reported to make up 22% (N=63) of the classified cell types (Onos et al., 2015). In addition to the larger percent of M1 cells found there was also a

variation that was not found in PFC; which reflects a higher level of firing rates (Figure 16 & 17).

Movement 2 (Figure 18 & 19)

Characterized by an increase in activity after the start and delay response that continues as the animal moves toward the sample and choice response and decreases just before a response is made. Unlike M1 cell there was no increase activity when approaching the base (non-reinforced levers). Movement 2 (M2) cells made up 7% (N=23) of all classified cells in central thalamus (Table 5). M2 cells are found in both MD (4%) and IL (9%) they were found in the more lateral portions of MD and in greater quantities in IL. M2 is one of the cell types first identified in PFC and was reported to make up 13% (N=5) of the classified cell types (Onos et al., 2015).

Post Reinforcement (Figure 20 & 21)

Is characterized by an increase in activity after reinforcement is delivered. The cell was named based on the event related timing but it is not believed to be reinforcement related. Timing analyses performed on cells found in PFC support the hypothesis that the cell is firing as the rat disengages from locations where reinforcement was delivered. Place tracking maps support this assessment as well (Figure 22). These cells were only found in one animal with electrode placement in IL and were clustered together (Figure 12). They made up a small percentage of the overall correlated cells (5% N=10). Based on the histological assessment they may have possibly been recorded from the lower portions of PC or the ventral lateral nucleus. Post reinforcement cells found in PFC (8% N=8) did not display the high firing rates found in the thalamic cells.

Reinforcement Suppression (Figure 23 & 24)

This cell type is characterized by a suppression during times of reinforcement however it does not appear to be related to the act of reinforcement but more the cessation of movement. These cells display a generally high rate of firing that drops drastically as the animal approaches and makes a response at reinforced locations. These cells are found in both MD (24% N=34) and IL (21% N=43). There is a sub-group of these cells that appear to code for lever location as well. This is seen as an increase in activity at the time of delay that corresponds to specific pairing of levers. For example, if the start/delay lever location was lever 3 the cell increases firing at the delay when the to be correct response is left to lever 2 or right to lever 4 (Figure 25 & 26). This can also be seen in the place tracking map (Figure 27). Reinforcement suppression cells were identified in PFC (N=3) however compared to central thalamus (N=77) there were fewer and they were not as robust in their firing rates.

The second group of cell types under the broad category of action is lever press related. These cells are identified by increases or decreases associated with the timing of lever presses. Very few of these cells were identified in central thalamus (4% N= 17) and the cell type lever press suppression found in PFC (2% N=5) was not found in any of the eight rats recorded. This cell type is characterized by a significant decrease in respond at the time of every lever response. In addition, although two cells were labeled as lever press excitation they did not meet the criteria set for this cell type in PFC.

Lever press excitation (Figure 28 & 29)

Lever press excitation (LPE) is a significant increase in activity at the time of every lever press. The two cell identified one in MD and one in IL only show significant increases in

activity at start, sample, and delay. Considering the proportion of LPE cell found in PFC (12% N=33) it may be that these cells are not the same.

Base lever responses (Figure 30 & 31)

Base lever responses are significant increases in activity that occurs only during start and delay responses. Four base lever cells were found two in MD and two in IL. Again this is a much smaller proportion than found in PFC (N=13). Interesting all four of the cells found in central thalamus only fire in response to one of the two possible base levers suggesting coding of location as well as the action (Figure 32).

Complex lever press (Figure 33 & 34)

This cell type is found only in IL (3% N=11) and is characterized by a brief but significant decrease in firing rate at the time of a lever press immediately followed by an increase in firing rate last about 0.5-1 s. This cell type was not found in PFC recordings.

The third group is preparatory. Two types of preparatory responses were found in PFC those that were characterized by an increase in firing rate before the start response (N=24) or an increase in firing rate before the delay response (N=19). Three of these cells were found in IL only in one animal (Figure 35 & 36).

The next group is delay-related activity, cells in this group display activity leading up to or during the delay. PFC recordings found a large number of delay excitation (N=40) although there were variations in the precise timing, all the cells showed significant increase in firing rate between the sample response and the delay response. This type of activity was not found in central thalamus. Two cells found in MD were identified as have a small but significant increase

in activity 1 s before the delay response that drops abruptly when the delay response is made (Figure 37 & 38).

Location/direction specific delay related (Figure 39 & 40)

This last cell type in delay related activity is specific to direction of movement as well as location. The cell type is characterized by a unique firing pattern that can be identified within the raster plots. The cell fires differentially for sample on some trials and correct choice on alternative trials. For example, the cell only fires from sample to delay for trials in which the sample lever is left and the to be correct response is lever 3. Subsequently, after the delay response it only fires for trial in which the correct response is left to lever 3. This can be best seen by the absence of firing seen before the 0 for left and after the 0 for right. In addition, when looking at reinforcement, which combines sample and correct responses, the sporadic raster plot fills in. Validating that the sample and correct responses are occurring on separate trials (Figure 39 & 40).

The second broad category is outcome. This is activity that is associated with reinforcement. There are three cells types identified: reinforcement excitation (RE), Anticipation, and Error. There was one error cell found in MD this cell type is an increase in activity only at the error response and no other significant firing (Figure 47 & 48). Error cells were also found in limited number in PFC (N=4).

Reinforcement excitation (Figure 41 & 42)

RE cells are identified as an increase in firing rate at the start of reinforcement delivery lasting throughout the 1.5-2 s that reinforcement is given. These cells were found in both MD (15% N=21) and IL (25% N=51) and were the second highest proportion of classified cells in

central thalamus (22% N=84). This was comparable to the proportion found in PFC (21% N=64). However, there was more variability in the duration and onset of excitation. Some cells fired only for the duration of the first pulse of reinforcement and other didn't excite until the second pulse was delivered. Although the timing was not consistent the increase in firing rate was significant and clearly associated with the delivery of reinforcement. Several of these cells were also directionally specific. For example, the cell only fires for right trials (Figure 43 & 44).

Reinforcement Anticipation (Figure 45 & 46)

Characterized by increased firing 0.5-1 s prior to the delivery of reinforcement that is also seen for error responses but falls off abruptly when reinforcement is not received. Cells that met this criteria were only found in IL (5% N=11). However, it appears as if some RE cells in MD begin to excite 200-300 ms prior to reinforcement delivery. It is unclear if this is an anticipatory response and may reflect a lag in information sent from PFC about the expectation of reinforcement therefore the excitation occurs later. Because they did not meet the criteria they were considered RE.

The last category of actions and outcomes consist of cells that fire in response to actions that result in no reinforcement as well as those that result in reinforcement. Action/Outcome cells (AO) are characterized as an increase in firing 1 s prior to making a lever response. For the base levers (start and delay) the activity drops abruptly when the lever is pressed. For reinforced levers the activity remains elevated throughout the reinforcement and for 0.5-1 s after the delivery of reinforcement has ended (Figure 49 & 50). These cells are only found in IL and make up 12% (N=25) of the classified cells found. Combination cells that were categorized as

LPE/RE were found in PFC but they were limited in number and eventually merged in with one of the two types. It is unclear if these are the same type of cells or if this is unique to thalamus.

Unclassified cells

There are two types of unclassified cells within the central thalamic data. First, unidentified behavioral correlates are cells that have significant increases or decreases in firing rate but did not correspond to the events of the task. Because we set the 99% PET criteria any cell that met that criteria and showed a pattern of activity was considered a correlated cell. Last are high activity cells, these are cells that fire at a high level (often double normal firing rates) and show a minimal (but significant) suppression. Because these cells all have well isolated clusters, solid waves forms and ISIs it seemed reasonable to categorize them as their own cell type.

DISCUSSION

The initial purpose of this study was to characterize cellular coding properties in central thalamus. Several hypotheses have been developed along the way. The first of these was to compare the current data to that collected in prefrontal cortex. Based on the strong reciprocal connections between central thalamus and PFC it was reasonable to expect to find overlap in the types of cells found. As Table 6 shows there is a great deal of overlap however there are several striking differences.

The lack of delay excitation found in central thalamus is one of these differences. The existing literature on this topic shows mixed results with delay related activity was identified in PFC and MD of non-human primates (Funahashi, Inoue, & Kubota, 1993; 1997; Wyder, Massoglia, & Stanford, 2003; 2004); however, studies using rats are not as clear. Many studies have identified delay related activity in PFC of rats similar to that found in non-human primates (Onos et al., 2015; Jung, et al., 1998; Baeg, et al., 2003) while others make the claim that it is not found in MD (Han et al., 2013). Studies that identified thalamic nuclei as 'driver' and 'modulatory' initially seemed to support the hypothesis that delay excitation would be found in MD. MD is the main 'diver' input into PFC via excitatory pathway and receives direct inputs from PFC (Shermann & Guillery 1998; 2011; 2012). This would suggest that delay related excitation in regions of PFC connected to MD would then trigger excitatory responses.

However, this does not appear to be the case, a better explanation may exist in evaluating the theory of higher order nuclei (Shermann & Guillery 1998; 2011; 2012). It is widely accepted that MD and IL are higher order nuclei but the interpretation of what this means varies. The

work of Sherman and Guillery claims that the role of higher order nuclei is to relay information between cortical areas. The question is what type of information is being relayed and how is it relevant to task performance?

Delay related activity is hypothesized to be involved in holding information online during the delay. Lesions to regions of PFC where delay related activity has been found have been shown to impair performance on tasks of working memory (Chudasama, et al., 2003; Chudasama, 2011; Uylings, Groenewegen & Kolb, 2003; Table 1). Lesions to MD have also been shown to impair performance in a delay dependent manor (Baily & Mair, 2005). It seems reasonable to infer based on these findings that the impairment was due to a disruption in the ability to hold information 'online' during the delay. But, if we consider that MD and IL receive efference copies (also referred to as corollary discharge) of motor commands being sent from PFC to brainstem motor centers this sheds a different light on the information MD is relaying to cortex (Shermann & Guillery, 2011; 2012; Sommer & Wurtz, 2008; Crapse & Sommer, 2008). The work on understanding corollary discharges stems from the study of our visual system (Sommer & Wurtz, 2008). In order for us to see unbroken, smooth images given the fact that our eyes are constantly moving the visual system requires information about current and planned eye movements are required as well as head and body movements. This concept can be applied to many types of planning. For example, an animal moving in the wild needs to be able to separate out sensory information from the environment verses sensory input caused by its own movements and actions in the environment (Sommer & Wurtz, 2008; Crapse & Sommer, 2008).

The prominent cell type found in MD is movement, with M1 and M2 making up nearly 50% of the total classified cells. Sommer and Wurtz (2008) suggest that the information being relayed to PFC is used to monitor our own movements. This hypothesis fits well with the data

collected and also explains commonalities of impaired performance on tasks. PFC impairment may well be due to an inability to hold information 'online' across a delay. However, an alternative explanation for delay dependent impairment with lesions to MD may be that PFC requires the relay from MD about current movements to adequately plan future actions. This does not explain the lack of delay related neurons in rats but not primates. One plausible explanation is task, primates are often restrained with limited mobility and most tasks require the animal to 'gaze' or they measure saccades. What is being interpreted as delay related activity could be found more readily because of the simpler motor responses being performed or the activity is more closely tied to the visual system and would not be seen in rodents with poor vision.

Central thalamus also displays a large number of cells characterized by suppression at the time of reinforcement. These cells have been grouped with movement related activity because the cell is firing except at times when the animal is known to not be in motion. This is a very general statement and more investigation into these cells in required. It was hypothesized that IL would display a greater amount of inhibitory responses which could be represented by suppression of activity. Unfortunately, these cells are found in both MD and IL, inhibition from basal ganglia is not a straightforward explanation. It is possible that they are in fact related to reinforcement and that the high levels of firing are arousal related due to input from the brainstem regions and the decrease in firing is a slight decrease in arousal while the animal consumes reinforcement. A systematic analysis of these cells including timing of the suppression and waveform width (which were conducted for the PFC cells) may provide more information.

Due to limited samples from each of the individual nuclei it is difficult to make evaluations of the point-to-point connections as previously proposed. However, there are commonalties that can be discussed. MDm was hypothesized to show cellular properties in relation to behavioral inhibition and learning and updating outcome expectancies (Table 1). Both of these can be connected to the efference copies discussed already, central thalamus not only receives efference copies of motor commands but also sensory information. The combination of motor planning and sensory feedback about reinforcement may aid in successfully completing these tasks. In addition, MDm was proposed to be primarily reinforcement related activity of the 28 cells in that region 21 of them are reinforcement related.

MDc and MDl were hypothesized to code for reward contingencies and reward value and movement (Table 1). The majority of cells recorded in these areas are movement related. This could be due to the limited success with implants aimed at MDc, further recording needs to be conducted with implants that hit the mark right through MDc before the reinforcement possibility can be discounted.

Interestingly CL and PC were hypothesized to show properties relevant to reward value and initiation of action sequences and this is where all the AO cells were recorded. Both of these areas are connected to anterior cingulate (ACC). A recent study conducted by Hayden and Platt (2010) recorded cells in ACC. Their results suggest that single cells in ACC can encode for actions and outcomes. They refer to this as multiplexing and found that a single cell would fire differentially to angle of eye movement (location) as well as value of reinforcement (low firing rate for low value; high firing rate for high value). This supports the hypothesis that IL may be more than just a relay and have more of a cognitive role in information being sent to PFC.

Although there were some commonalities in cell types between MD and IL there were more distinct differences. The majority of the cells found in IL appeared to code for more complex aspects of the task such as: reinforcement cells that only fire for right correct trials, the AO cells that appear to be coding for both actions and outcomes, the complex lever press cells that may be signaling the start of each phase in the task, base lever responses that fire only for one of the two base levers location and the location/direction specific cells.

In general, the results support the hypothesis that due to the reciprocal projects from MD and IL to PFC recordings from central thalamus will contain similar cells types to those found in PFC. However, it was expected that MD would contain delay-related activity similar to that found in PFC which was not found. The high percentage of movement-related and reinforcement activity found in MD is consistent with the associated behavior (Table 1). Goal directed behavior for this task is defined by movement from one lever location to the next. It is possible that increases in activity during movement could be related to goal directed behavior and action planning. By far the most interesting finding is the possible role of IL, once thought to mainly be involved in attention and arousal aspects of behavior. The results found in this study shed a very different light on the role of IL. Cells recorded in IL appear to be coding more complex aspects of the task than the cell types found in MD.

Future directions for this data would be to conduct the timing analysis of all cells so they can be better compared to PFC. In addition, the recent waveform width analysis performed in our lab may provide more information about the cells that seem to be unique to IL. Lastly, my initial evaluation was that thalamus did not produce context specific data like PFC, however, I was incorrect. My initial assessment was based on the first few animals recorded with implants located in MD. MD does not appear to code for much context, but a large portion of cells in IL

do appear to code for context specific information. The systematic evaluation of all MD and IL place maps should be conducted to quantify this difference.

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APPENDIX A

Table 1:

Reciprocal connections between MD and PFC and associated behavior.

	Sub-Nucleus	**PFC***	**Behavior***
MD	MDm	Prelimbic, Infralimbic, Orbital, & Anterior Cingulate	Learning and updating outcome expectancies, Retention of information across delay, Behavioral inhibition, Reward contingencies, & Reward Value
	MDc	Orbital & Agranular Insular	Reward contingencies, Reward value – Delay related
	MDl	Orbital, Anterior Cingulate, FR2, & Agranular Insular	Reward contingencies, Reward value – Delay related, Speed of responding, & Initiation of action sequences

* Not all inclusive

Table 2: Reciprocal connections between IL and PFC and associated behavior.

	Sub-Nucleus	PFC*	Behavior*
IL	CL	Somatosensory, Motor (M1 & FR2), Anterior Cingulate	Reward value, Speed of responding & initiation of action sequences
	PC	Anterior Cingulate & FR2	Reward value & Initiation of action sequences
	CM	Prlimbic, Agranular Insular, Primary Motor, Gustatory & Visceral	Retention of information across delay, Reward value, Speed of responding, & initiation of action sequences

* Not all inclusive

Table 3: List of TTL pulses and the behavioral event, including the Z-pulse used to trigger the TTL from Med-PC.

TTL Pulse	**Behavioral Event Marked**	**Programed Z Pulse**
1	START = start response (sample lever out)	Z4
2	SAMPLE = sample response (reinforcement, start of delay)	Z5
3	DELAY = end of delay response	Z6
4	CHOICE = response at end of delay (combination of correct and error)	Z7 OR Z8
5	CORRECT = correct choice (reinforcement received)	Z7
6	ERROR = incorrect choice	Z8
7	LEV1 = end of delay response when the 'to be correct' response is LEV1	Z10
8	LEV2 = end of delay response when the 'to be correct' response is LEV2	Z11
9	LEV3 = end of delay response when the 'to be correct' response is LEV3	Z12
10	LEV4 = end of delay response when the 'to be correct' response is LEV4	Z13
11	LEFT = end of delay response when the 'to be correct' response is left	Z14
12	RIGHT = end of delay response when the 'to be correct' response is right	Z15
13	REINFORCEMENT = 2nd pulse of water received (1s after the first delivery of reinforcement)	Z9

Table 4: Electrode Coordinates

RAT #	AP (FROM IA)	ML	DV START (FROM IA)	DV END (FROM IA)
1 (MDm)	6.7	0.5	5.2	2.8
2 (MDl)	6.7	1.0	5.4	3.4
3 (IL)	6.7	1.2	5.3	3
4 (IL)	6.2	1.3	5.2	3.2
5 (IL)	5.7	1.4	4.7	2.7
6 (LEFT; MDc)	6.2	0.8	5.2	3.4
6 (RIGHT; MDm)	6.2	0.2	5.2	3.4
7 (LEFT) **Placement is all in Right Hemesphere**	7	0.2	6.1	4.4
7 (RIGHT) **Placement is all in Right Hemesphere** **Target location** **Histology not completed**	7	0.6	6.1	4.4
8 (DUAL) **Data analyzed histology not completed**	6.2	0.6	5.4	XX
9 (DUAL) **Data not yet analyzed**	6.2	0.6	5.4	XX

Table 5: MD-IL Cell types and counts

MD Cell Types and Counts Actions		
Movement Related	**101**	**70%**
Movement 1	62	44%
Movement 2	5	4%
Post Reinforcement	0	0%
Suppression at Reinforcement	34	24%
Lever Press Related	**3**	**2%**
Excitaion	1	1%
Base	2	1%
Complex	0	0%
Preparatory	0	0%
Start	0	0%
Delay	0	
Delay related	**2**	**1%**
Location/Direction Specific Movement	0	0%
Delay Response	2	1%
Outcomes		
Reinforcment Related	**22**	**15%**
Anticipation	0	0%
Excitation	21	15%
Error	1	1%
Combinations		
Action/Outcome	0	0%
Total Classified Cells	128	89%
Unclassified	**16**	**11%**
Unidentified Correlate	12	
High Activity	4	
All Corrolated Cells	144	25%
Total Uncorrelated Cells	426	
Total Cells	570	43%

IL Thalamic Cell Types and Counts Actions		
41%	**100**	**Movement Related**
14%	29	Movement 1
9%	18	Movement 2
5%	10	Post Reinforcement
21%	43	Suppression at Reinforcement
6%	**14**	**Lever Press Related**
0%	1	Excitaion
1%	2	Base
5%	11	Complex
1%	3	Preparatory
	3	Start
	0	Delay
6%	**14**	**Delay related**
7%	14	Location/Direction Specific Movement
0%	0	Delay Response
Outcomes		
26%	**62**	**Reinforcment Related**
5%	11	Anticipation
25%	51	Excitation
0%	0	Error
Combinations		
12%	25	Action/Outcome
90%	218	Total Classified Cells
10%	**23**	**Unclassified**
	12	Unidentified Correlate
	11	High Activity
32%	241	All Corrolated Cells
	524	Total Uncorrelated Cells
57%	765	Total Cells

Table 6: Cell counts for all central thalamic recordings compared to cell types reported to be found in PFC (Onos et al., 2015)

Thalamic Cell Types and Counts Actions			PFC Cell Types and Counts		
Movement Related	**201**	**52%**	**29%**	**87**	**Movement Related**
Movement 1	91	26%	22%	63	Movement 1
Movement 2	23	7%	5%	13	Movement 2
Post Reinforcement	10	3%	3%	8	Post Reinforcement
Suppression at Reinforcement	77	22%	1%	3	Suppression at Reinforcement
Lever Press Related	**17**	**4%**	**17%**	**51**	**Lever Press Related**
Excitaion	2	1%	12%	33	Excitaion
Base	4	1%	5%	13	Base
Complex	11	3%	2%	5	Suppression
Preparatory	3	1%	14%	43	Preparatory
Start	3	1%	8%	24	Start
Delay	0		7%	19	Delay
Delay related	**16**	**4%**	**13%**	**40**	**Delay related**
Location/Direction Specific Movement	14	4%	14%	40	Delay excitation
Delay Response	2	1%	0%		
Outcomes					
Reinforcment Related	**84**	**22%**	**21%**	**64**	**Reinforcment Related**
Anticipation	11	3%	6%	18	Anticipation
Excitation	72	21%	15%	42	Excitation
Error	1	0%	1%	4	Error
Combinations					
Action/Outcome	25	7%			
Total Classified Cells	346	90%	96%	285	Total Classified Cells
Unclassified	**39**	**10%**	**4%**		**Unclassified**
Unidentified Correlate	24			13	Unidentified Correlate
High Activity	15				
All Corrolated Cells	385	29%	33%	298	All Corrolated Cells
Total Uncorrelated Cells	950			602	Total Uncorrelated Cells
Total Cells	1335			900	Total Cells

APPENDIX B

Figure 1: Thalamo-Cortico-Striatal Circuit

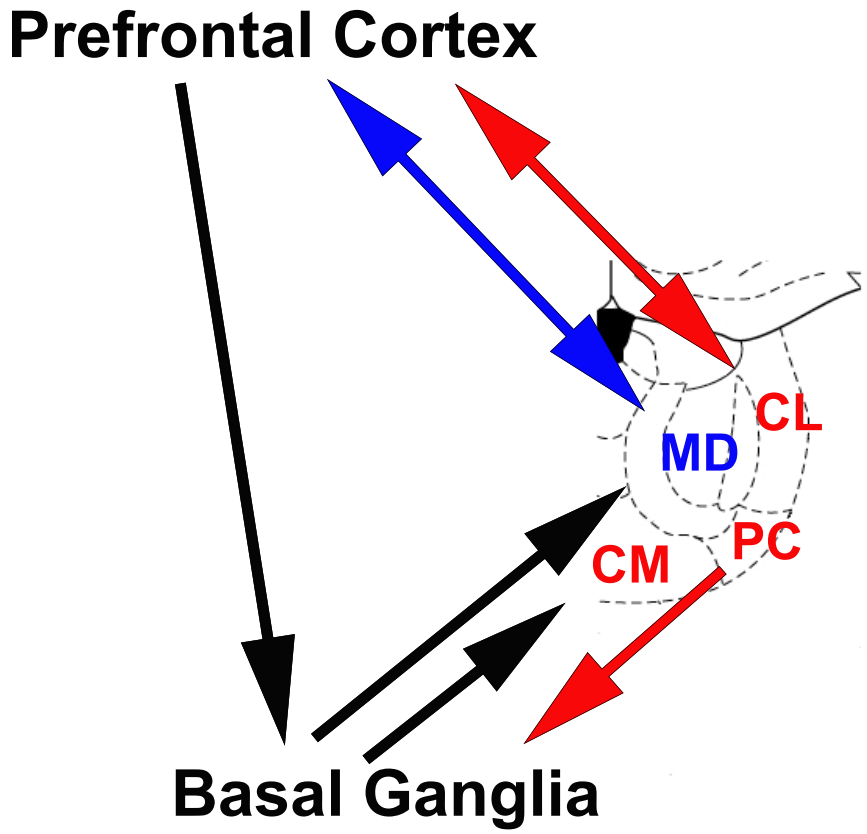


Figure 2: Recording apparatus

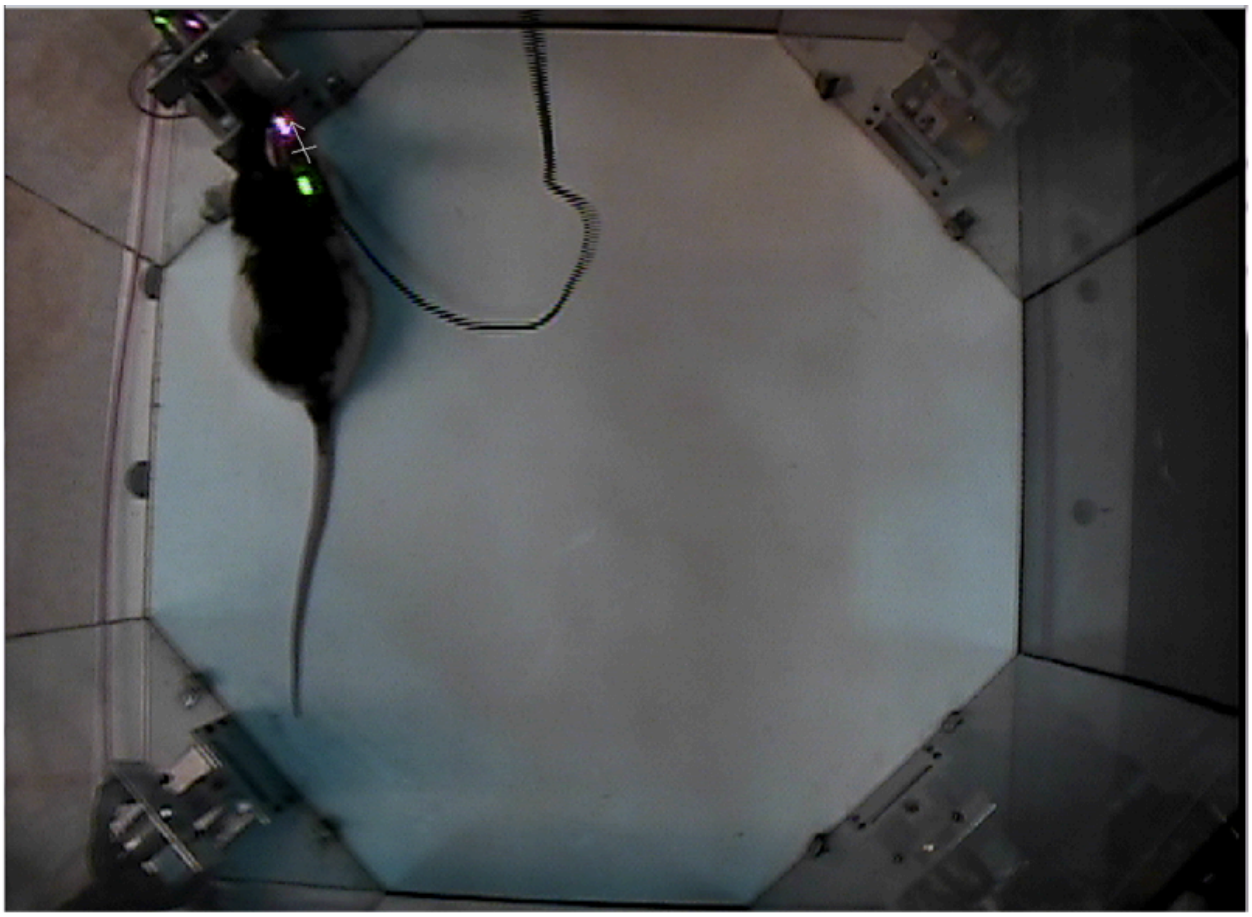


Figure 3: DNMTP Task

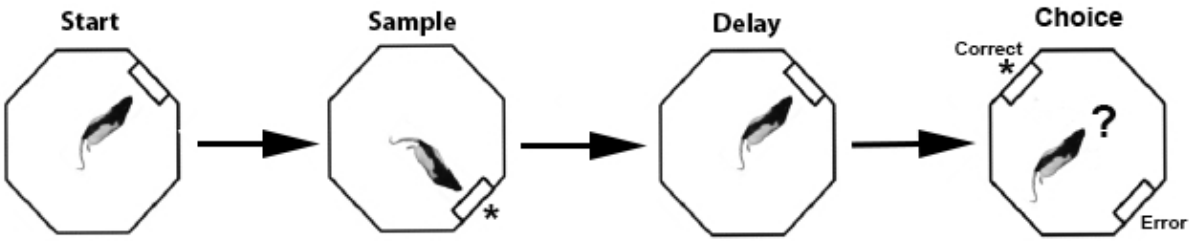


Figure 4: 3D spike sort example cluster, wave form and ISI

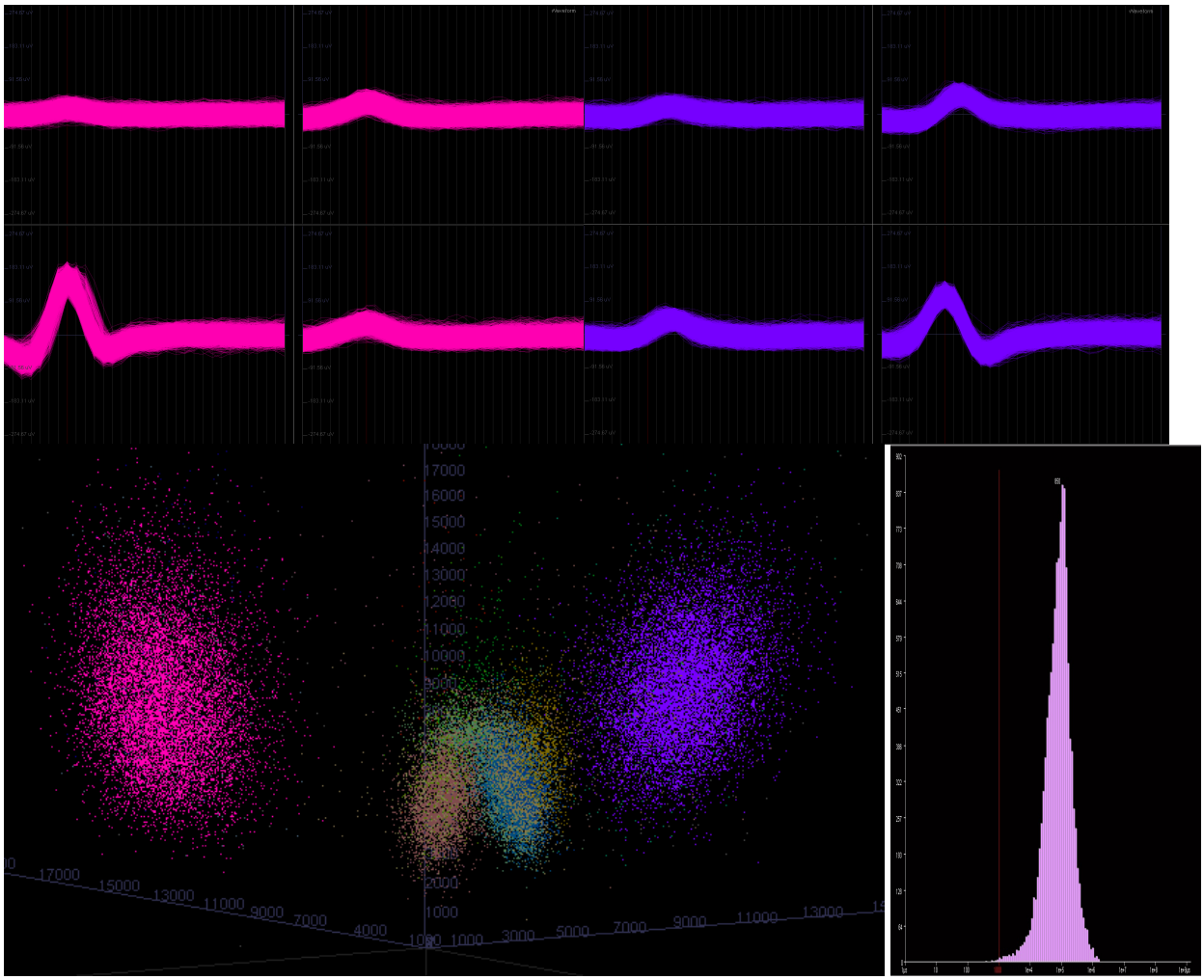
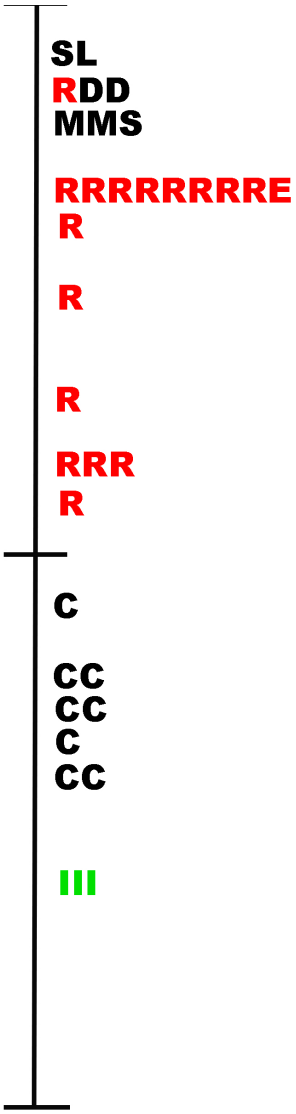


Figure 5: Rat 1 Electrode track with Cell types



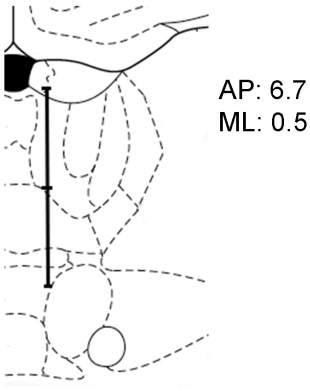
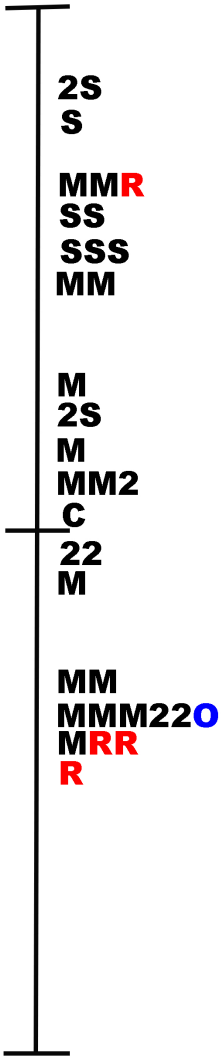


Figure 6: Rat 3 Electrode track with Cell types



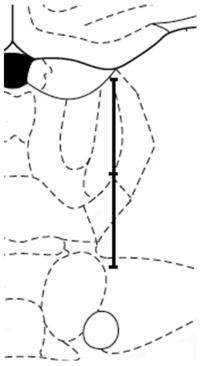
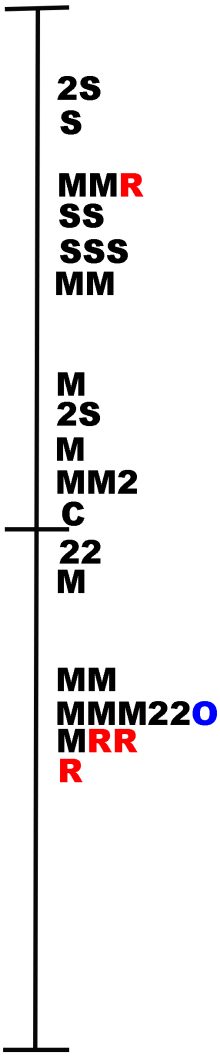


Figure 7: Rat 2 Electrode track with Cell types



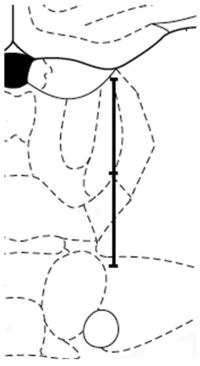


Figure 8: Rat 6 Electrode track with Cell types

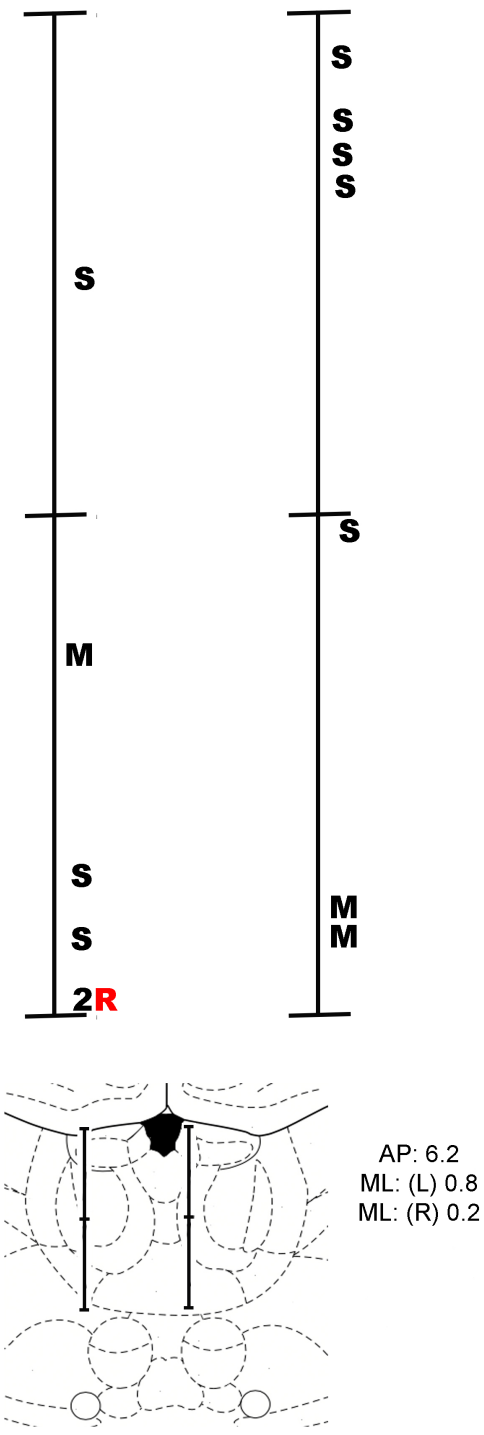


Figure 9: Rat 7 Electrode track with Cell types

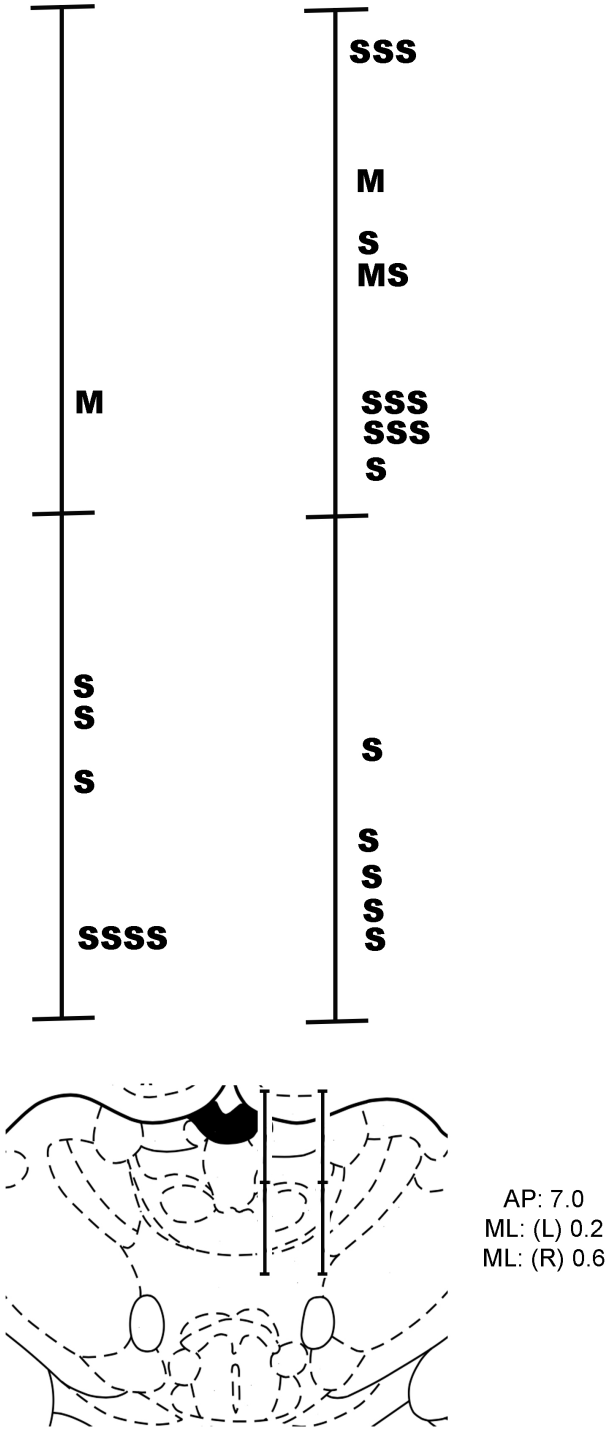


Figure 10: All MD Electrode track with Cell types

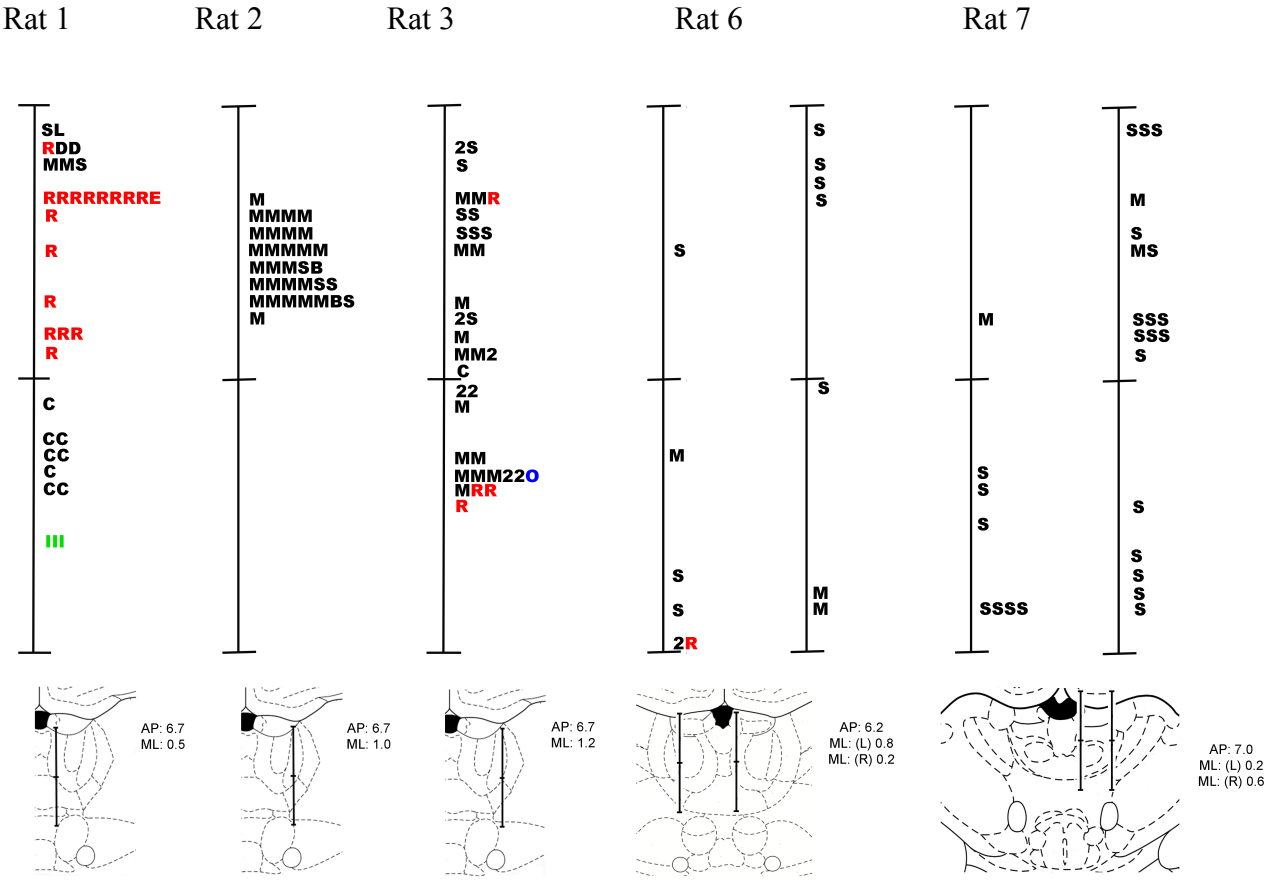


Figure 11: Rat 4 Electrode track with Cell types

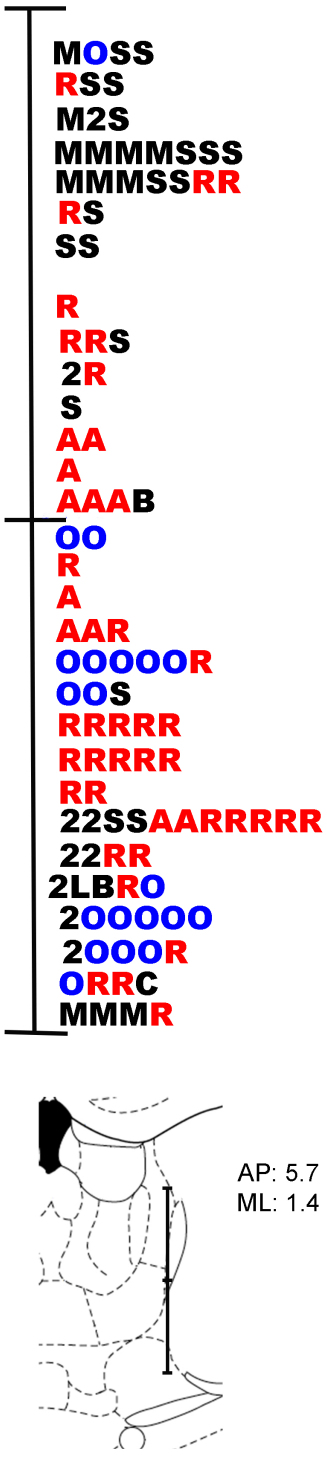


Figure 12: Rat 5 Electrode track with Cell types

Figure 13: All IL Electrode track with Cell types

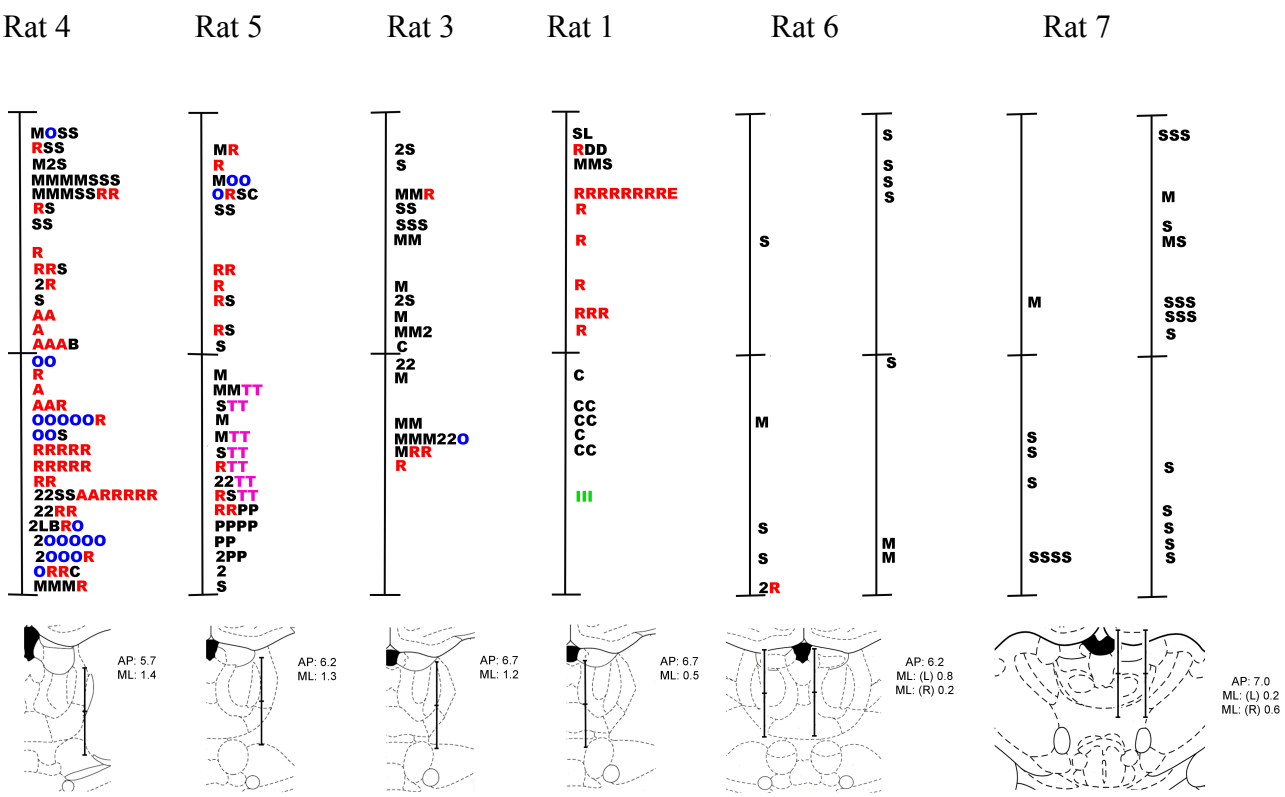


Figure 14: Movement 1 cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

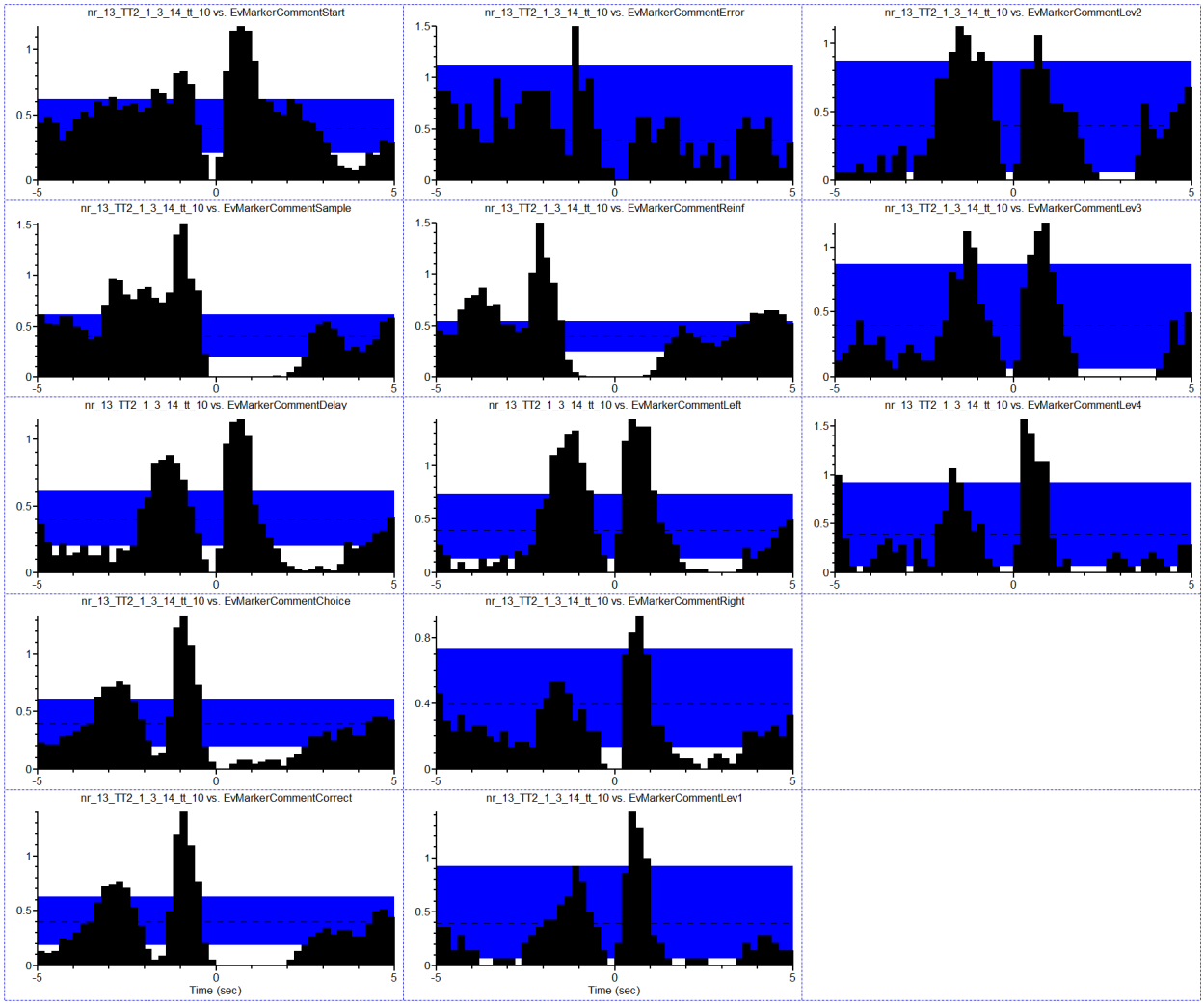


Figure 15: Movement 1 cell peri-event histogram with raster plots

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

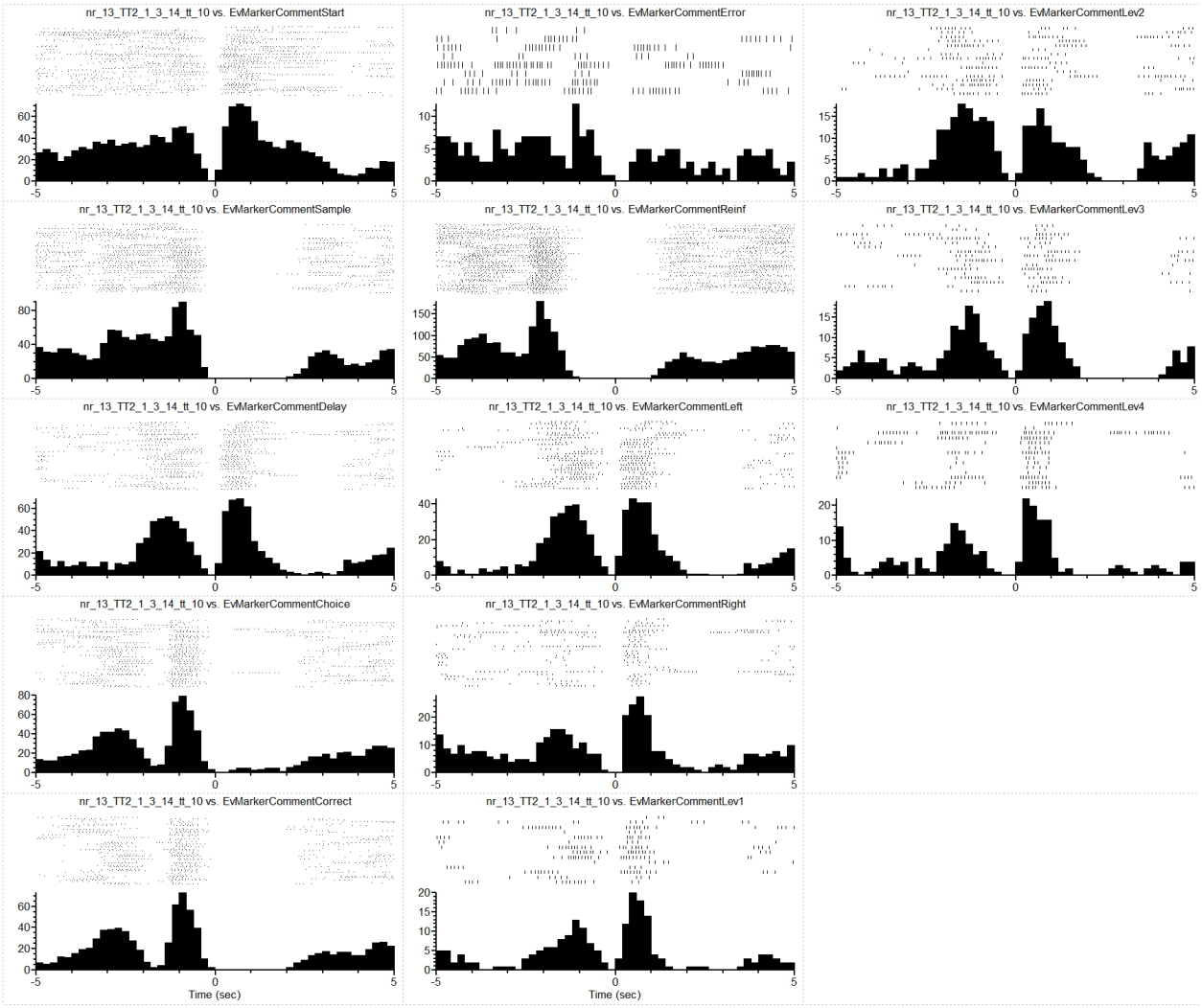


Figure 16: High activity Movement 1 cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

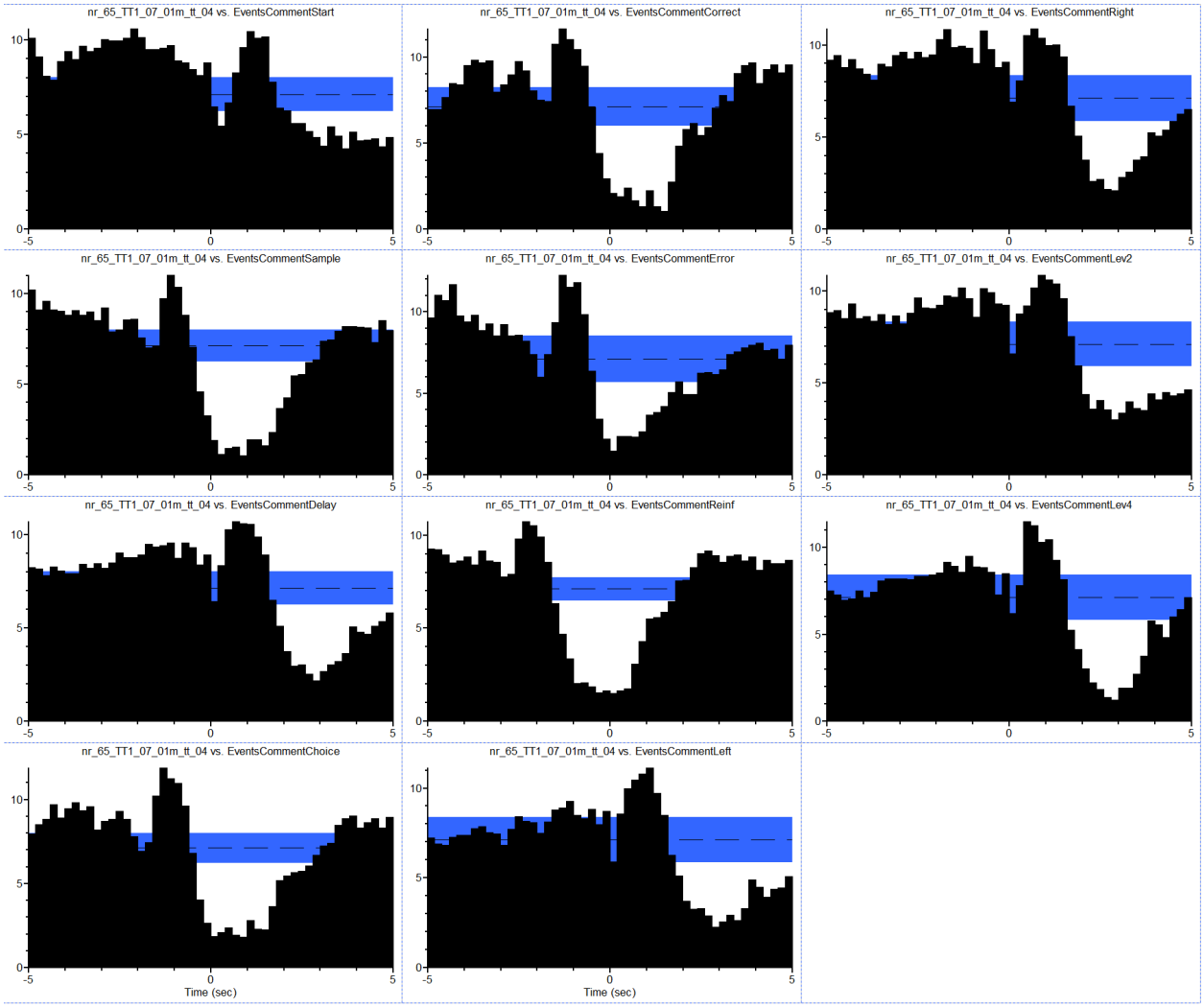


Figure 17: High activity Movement 1 cell peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

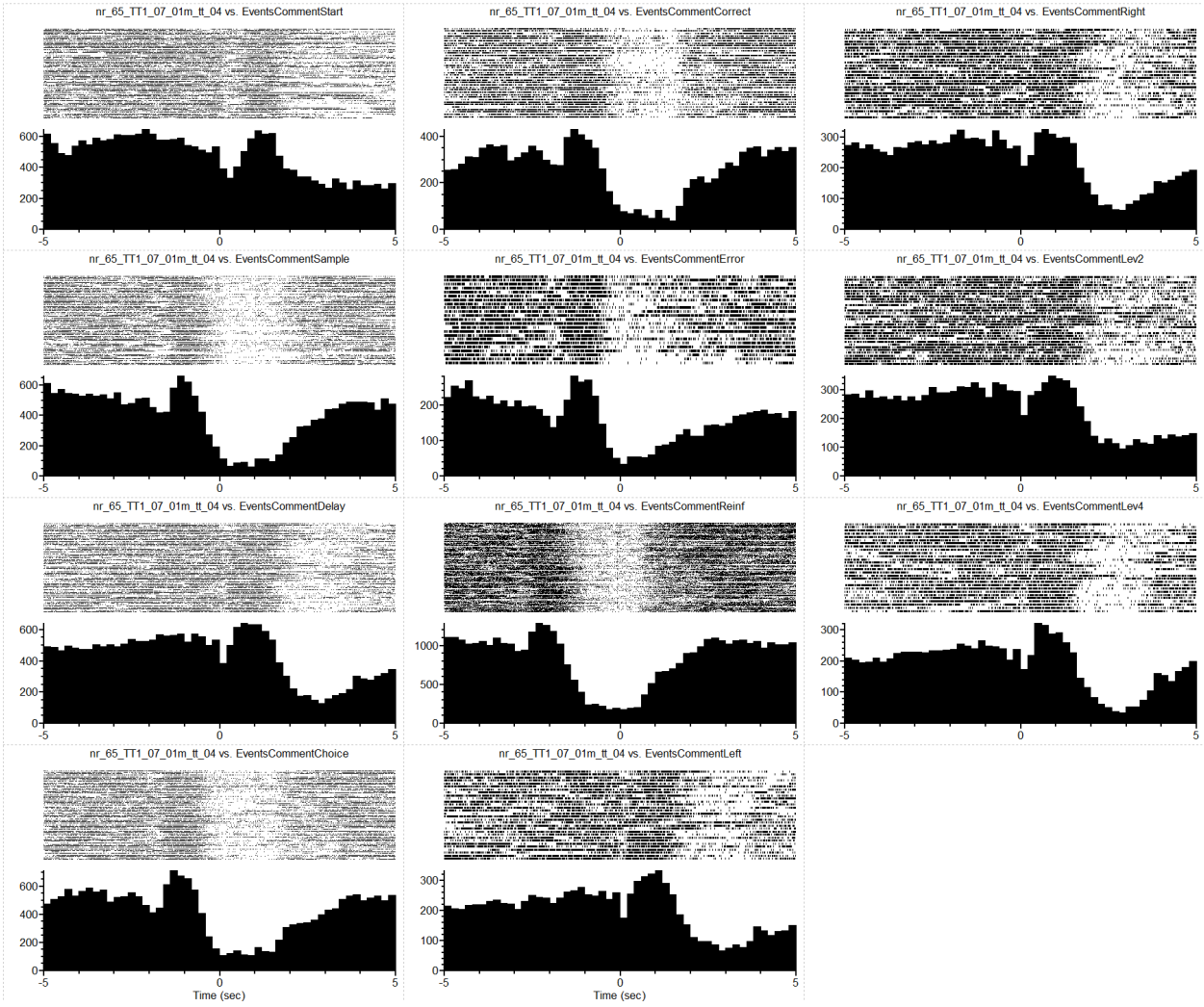


Figure 18: Movement 2 cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

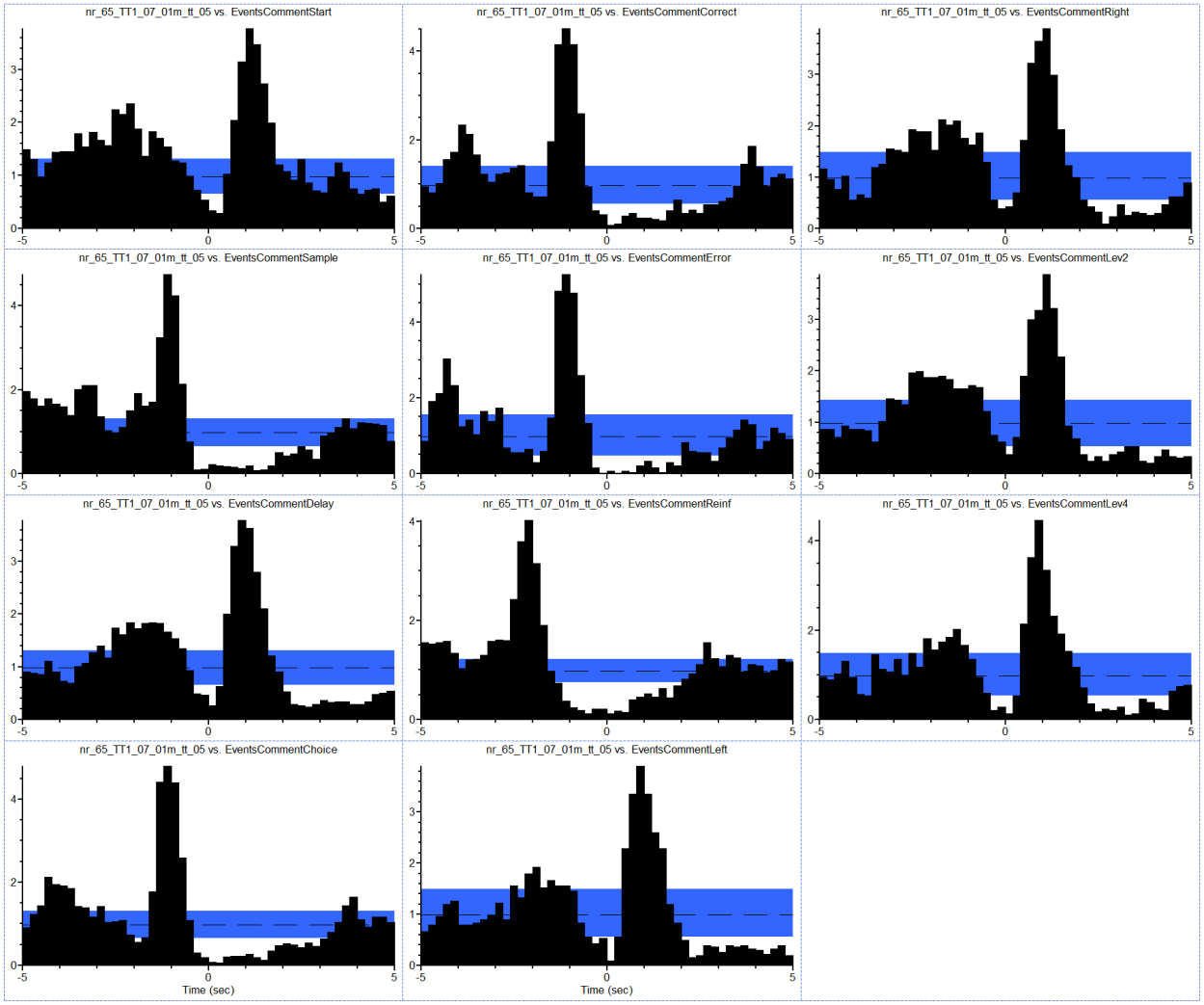


Figure 19: Movement 2 cell peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

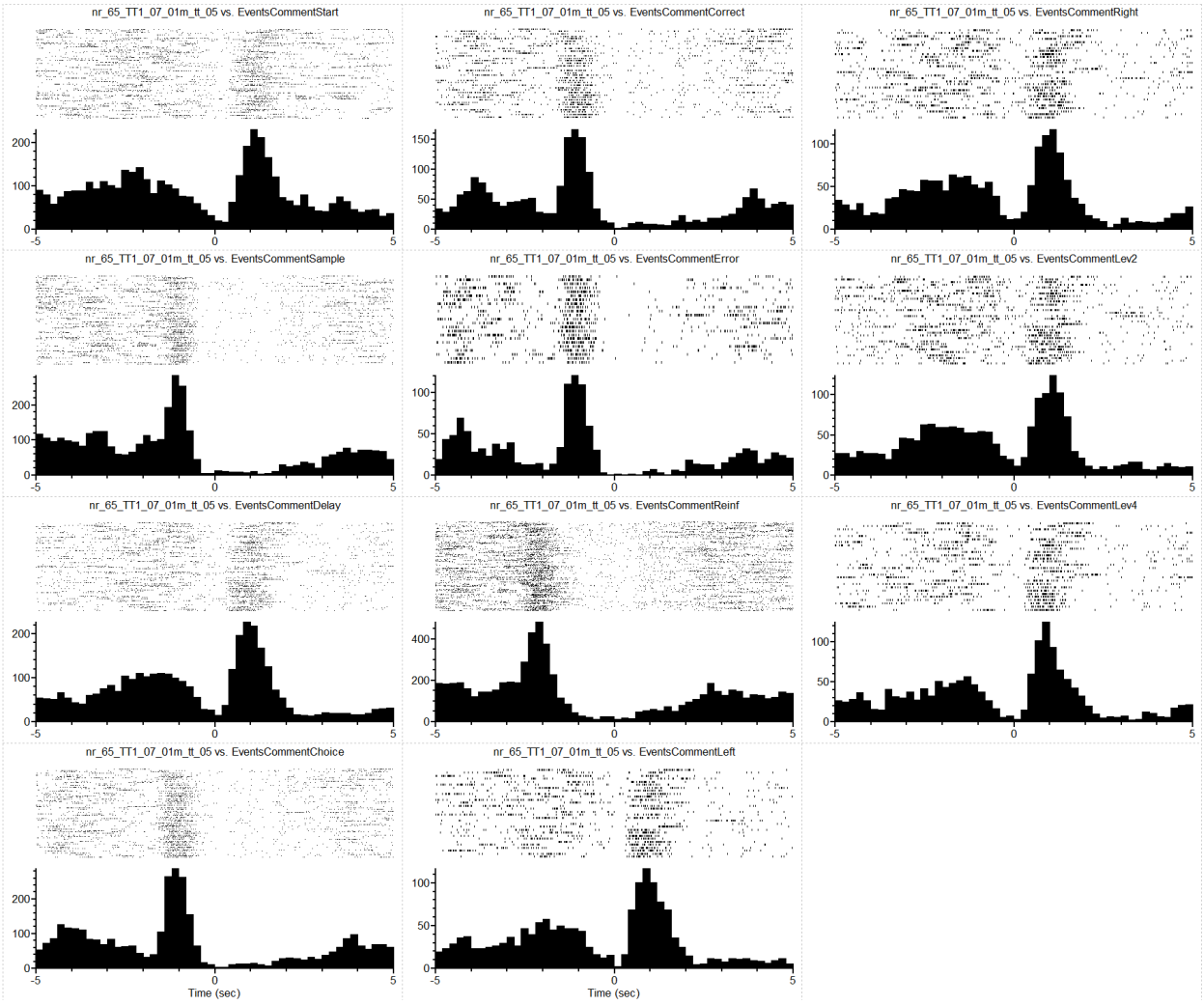


Figure 20: Post Reinforcement cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 1, Lever 3

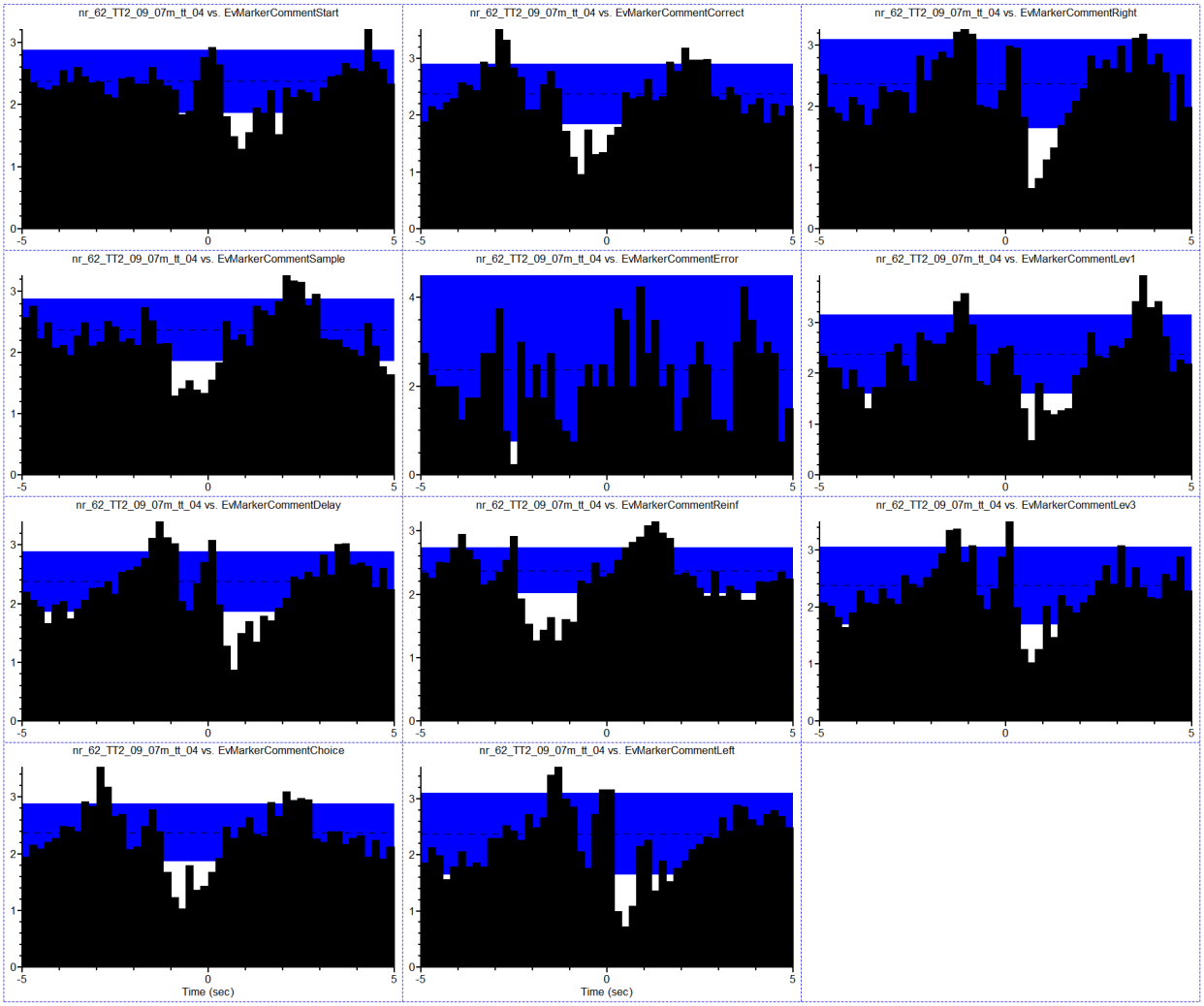


Figure 21: Post Reinforcement cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Reinforcement, Right, Left. Third Column: Error, Lever 1, Lever 3

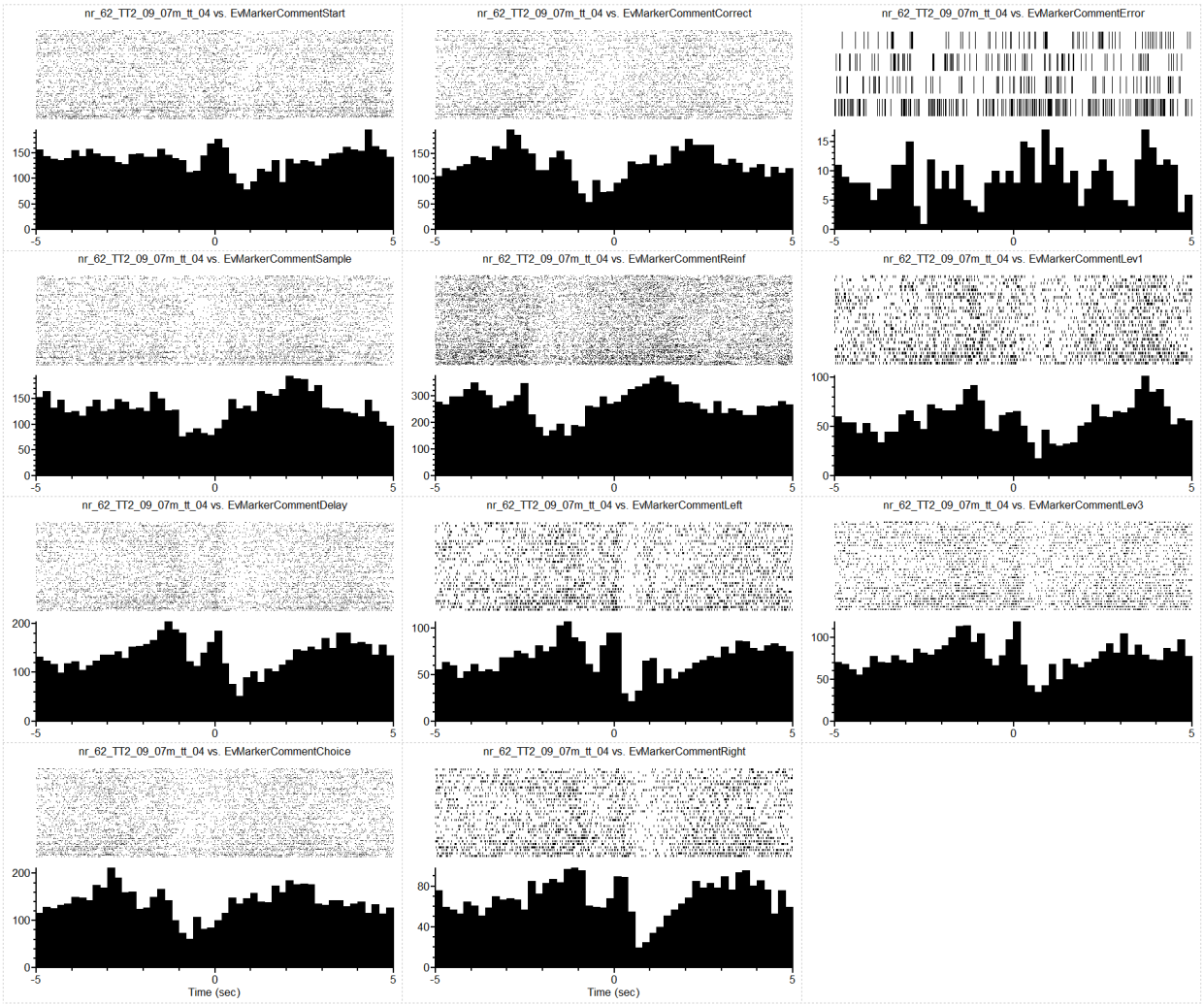


Figure 22: Post Reinforcement cell video tracking map

Top left corner is a reinforced lever location, the path of highest activity can be see to the left and right of the lever location.

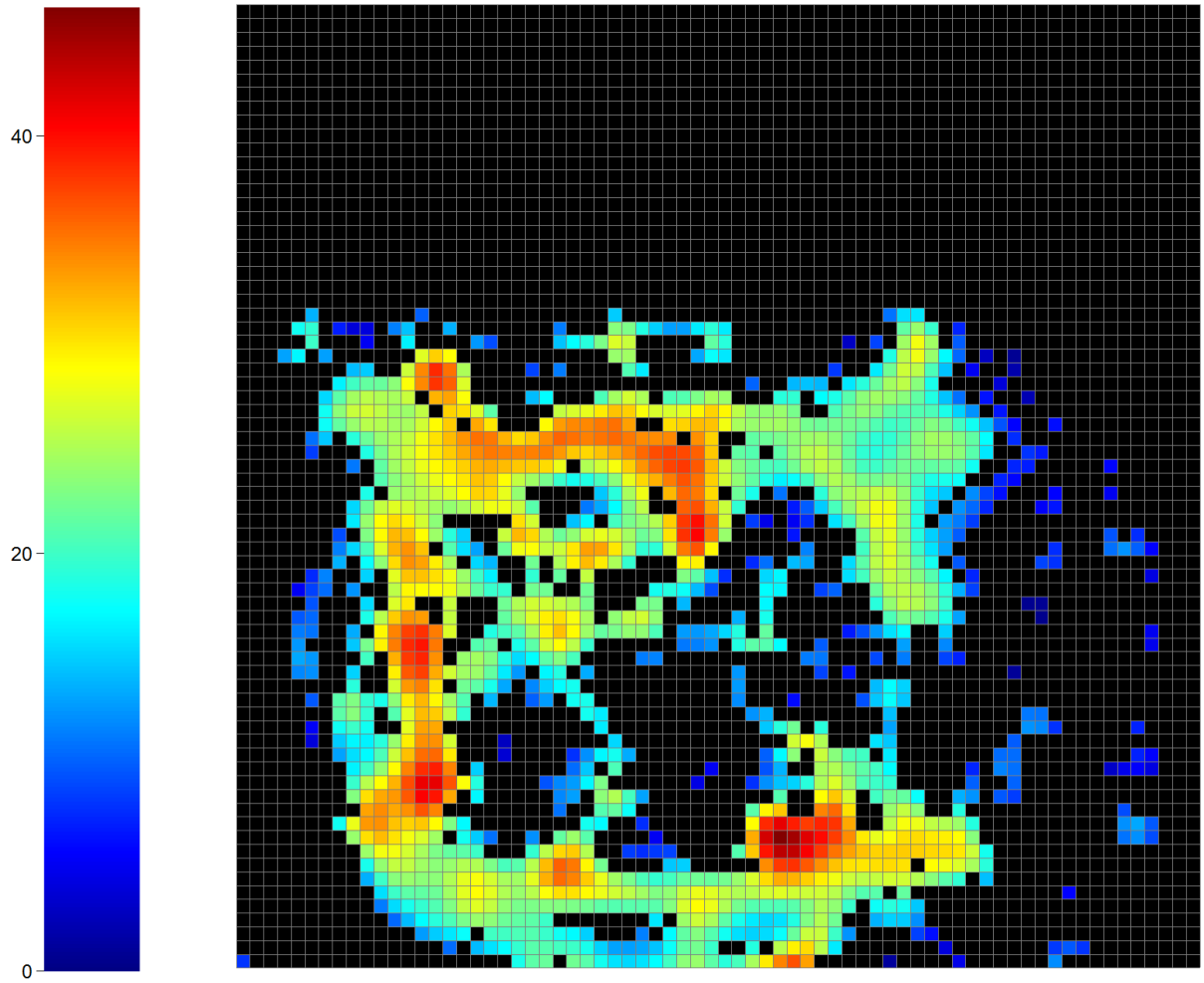


Figure 23: Reinforcement Suppression cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

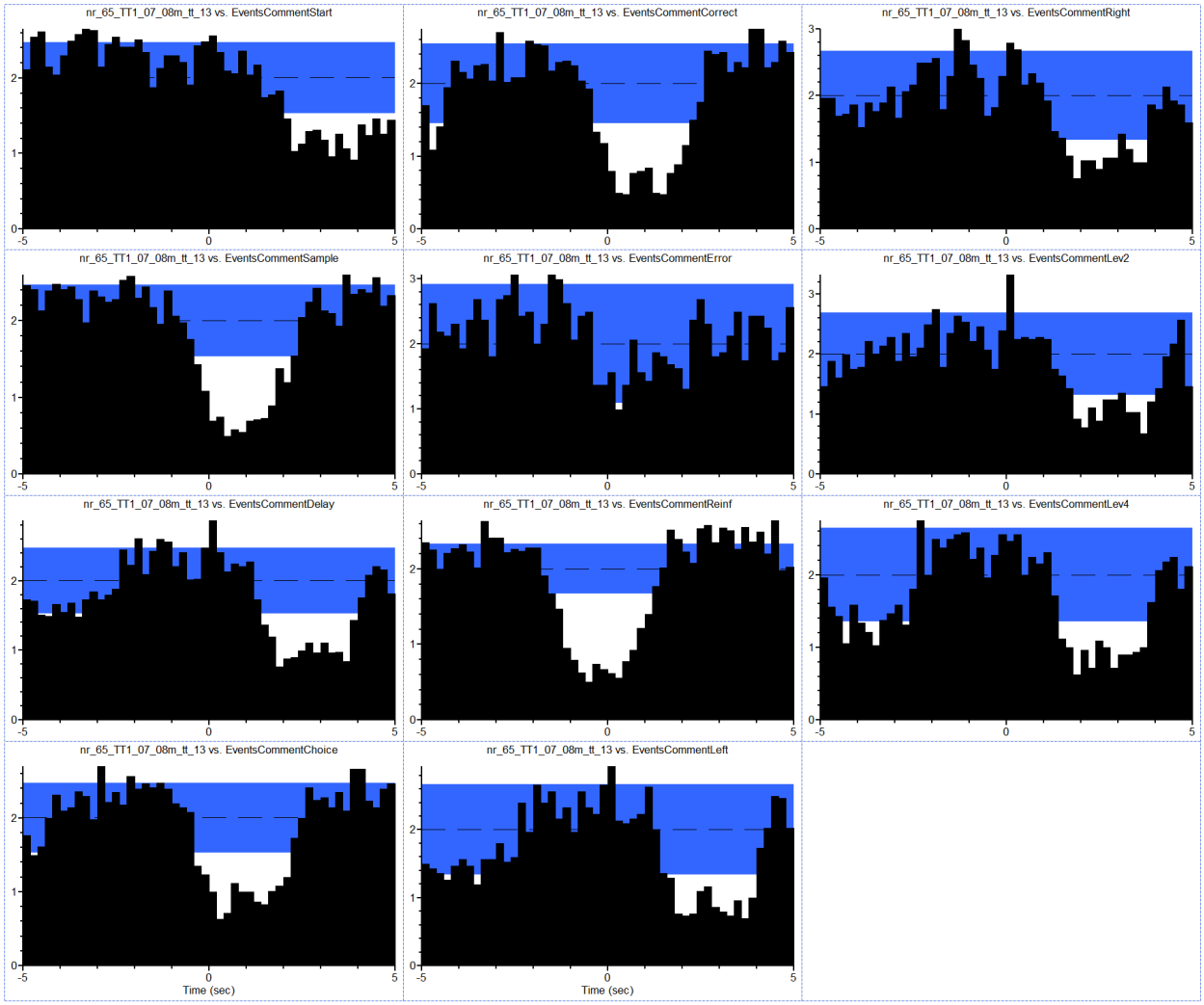


Figure 24: Reinforcement Suppression cell prei-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

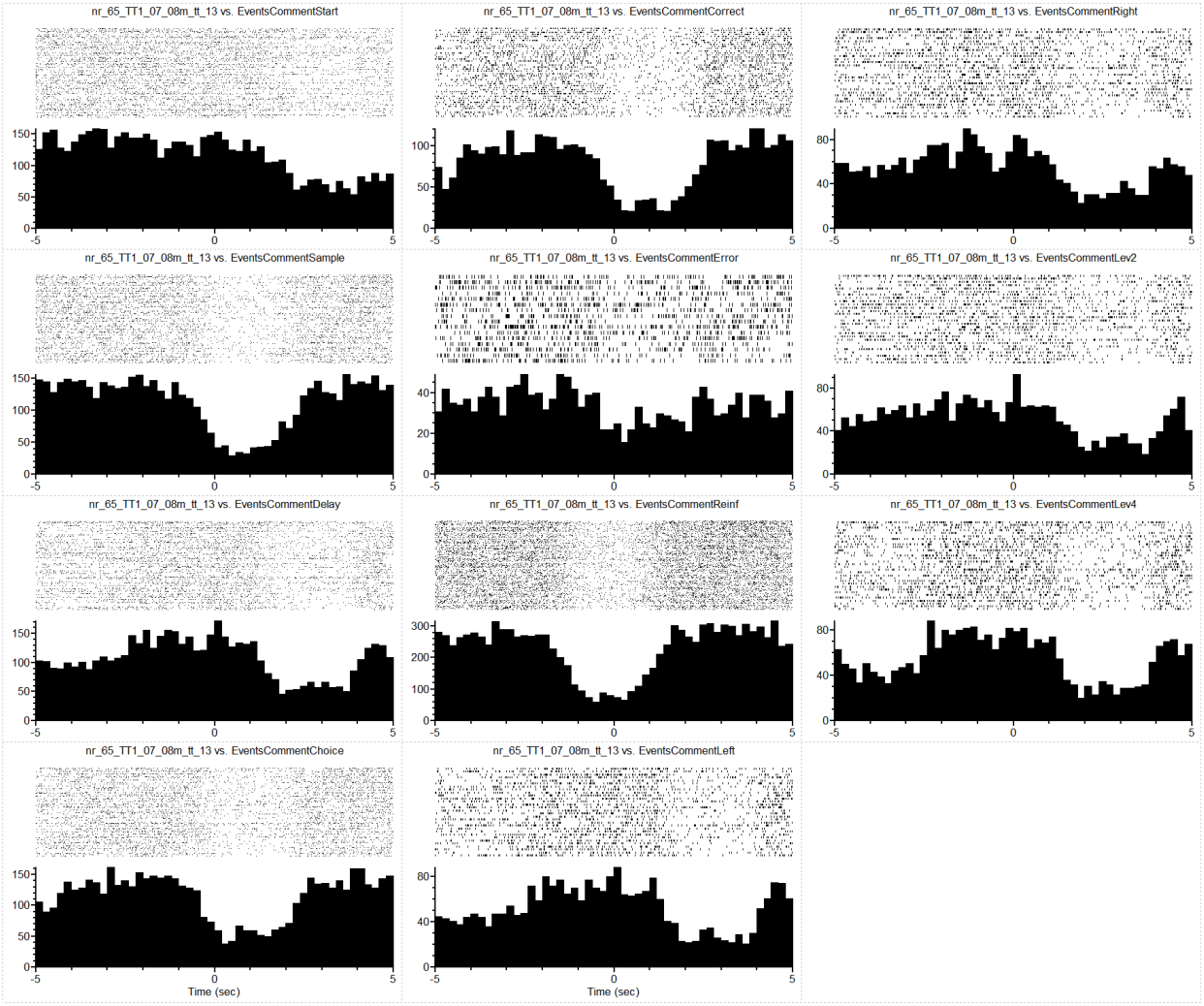


Figure 25: Delay related - Reinforcement Suppression cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

An increase in activity at the time of the delay response can be seen to correspond to matching patterns of activity on left & lever 2 as well at right & lever 4.

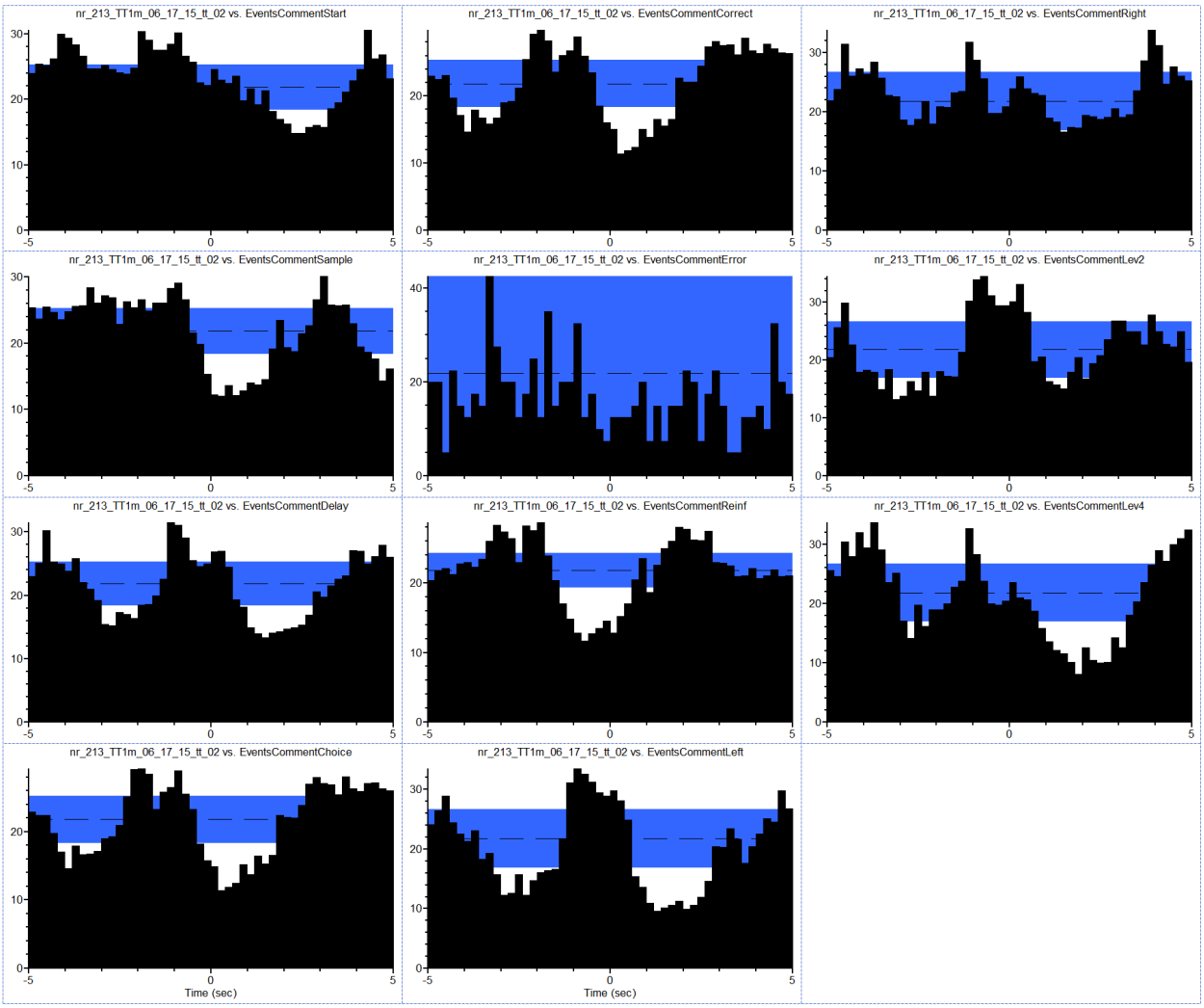


Figure 26: Delay related - Reinforcement Suppression cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

An increase in activity at the time of the delay response can be seen to correspond to matching patterns of activity on left & lever 2 as well at right & lever 4.

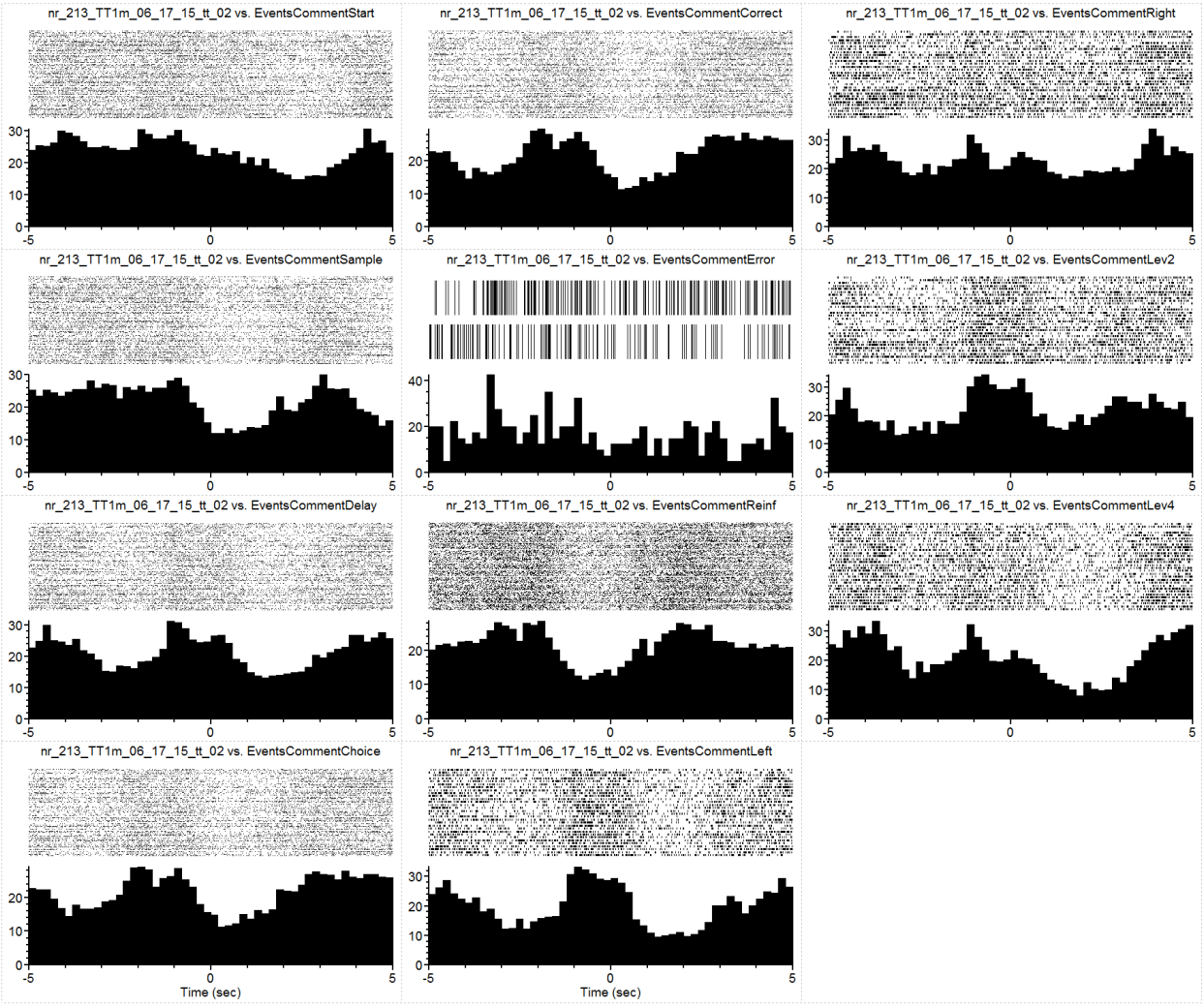


Figure 27: Delay related - Reinforcement Suppression cell video tracking map

An increase in activity at the time of the delay response can be seen to correspond to matching patterns of activity on left & lever 2 as well at right & lever 4. Top right (lever 3) and lower left (lever 1) levers are a delay responses. Highest activity can be seen at lever 3 which corresponds to the delay response when the to be correct response is left to lever 2 or right to lever 4.

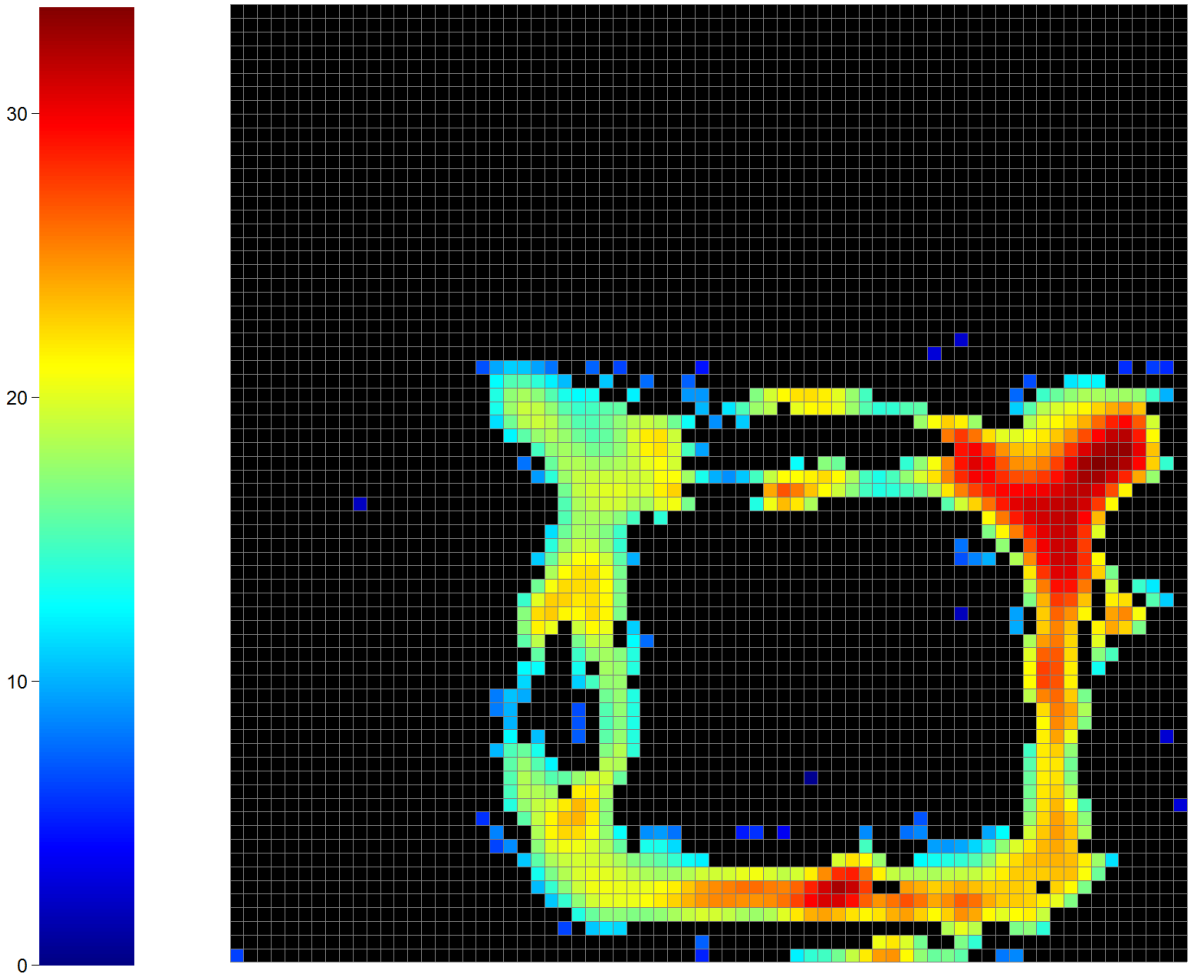


Figure 28: Lever press excitation cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

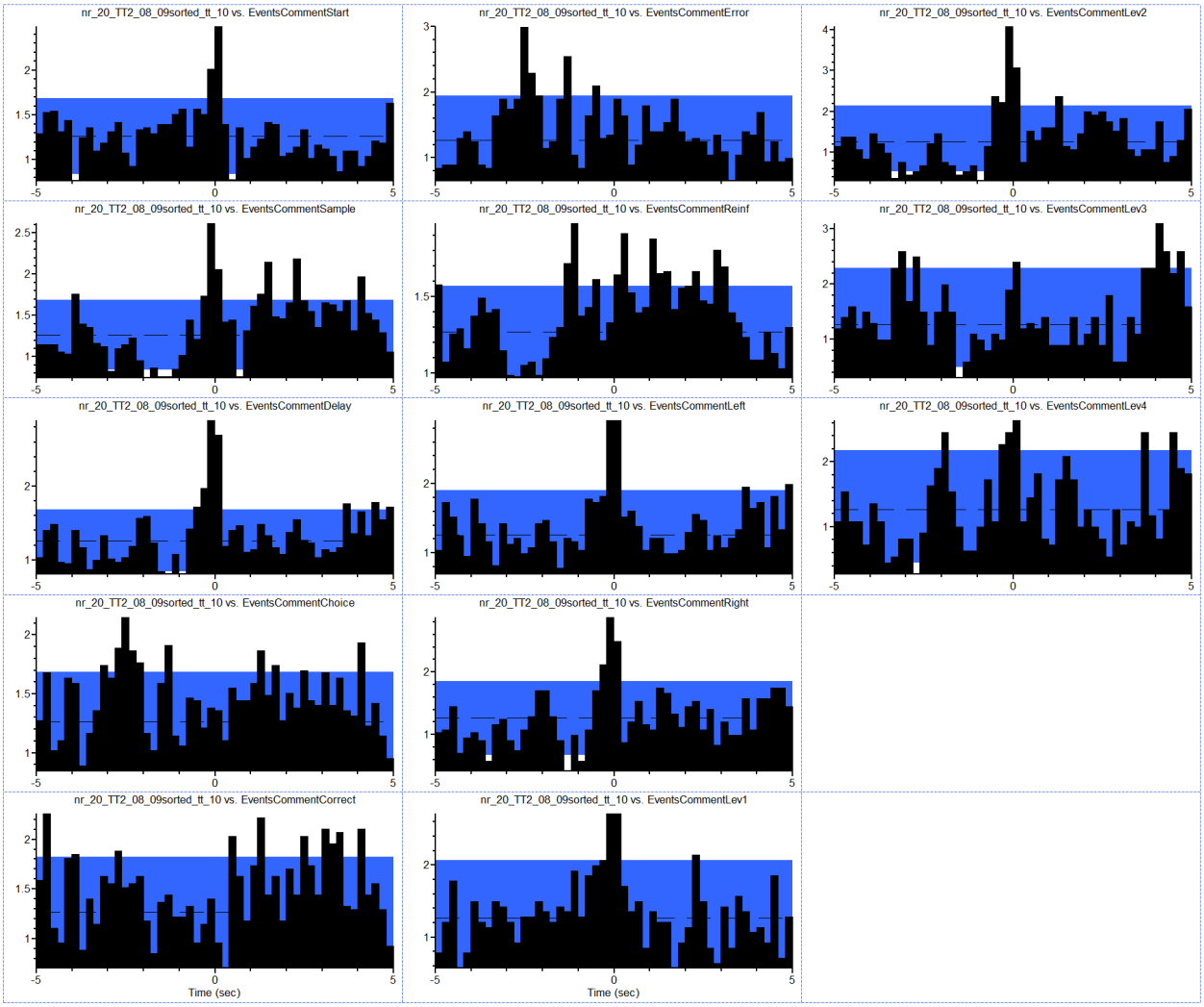


Figure 29: Lever press excitation cell peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

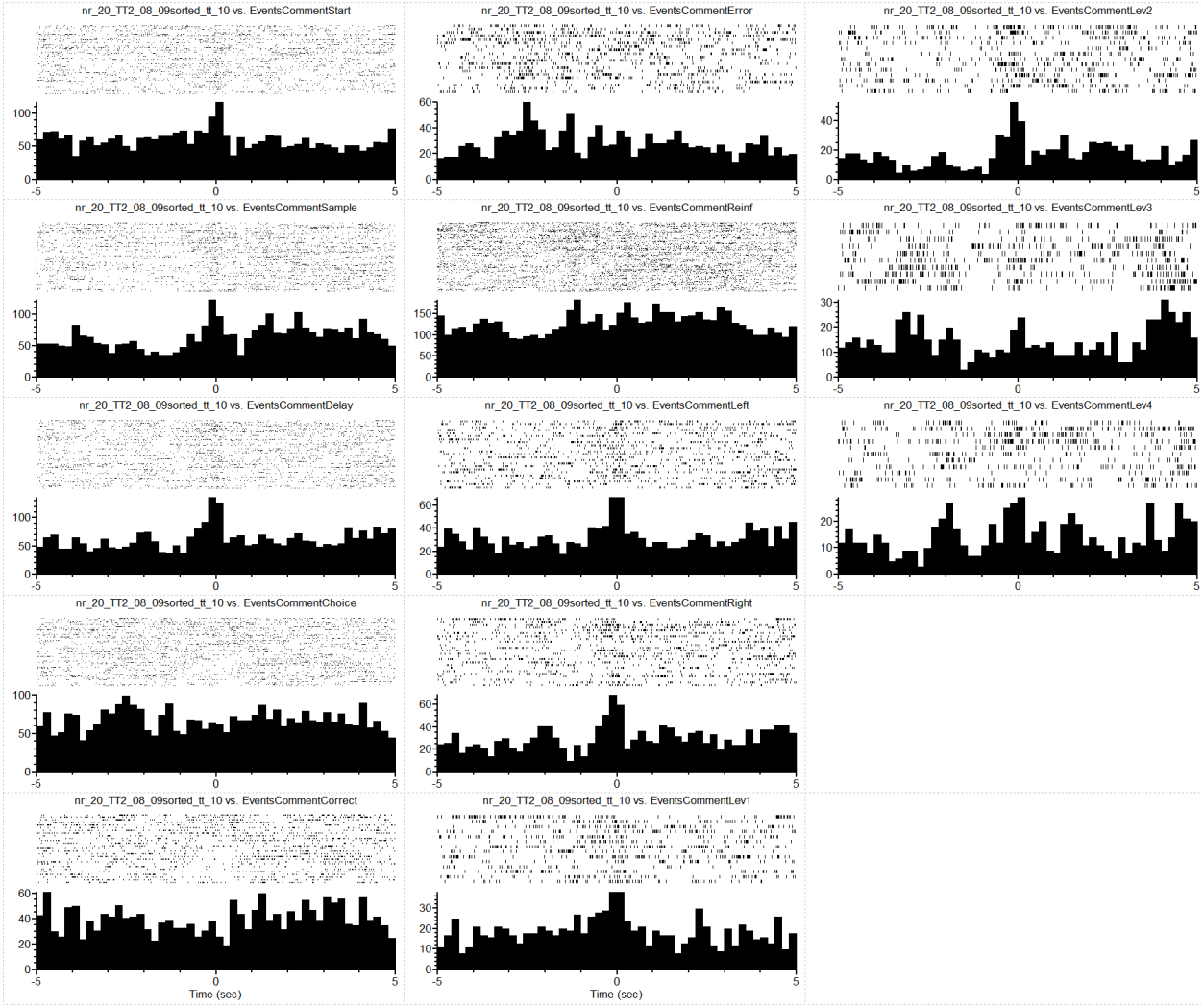


Figure 30: Base lever cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

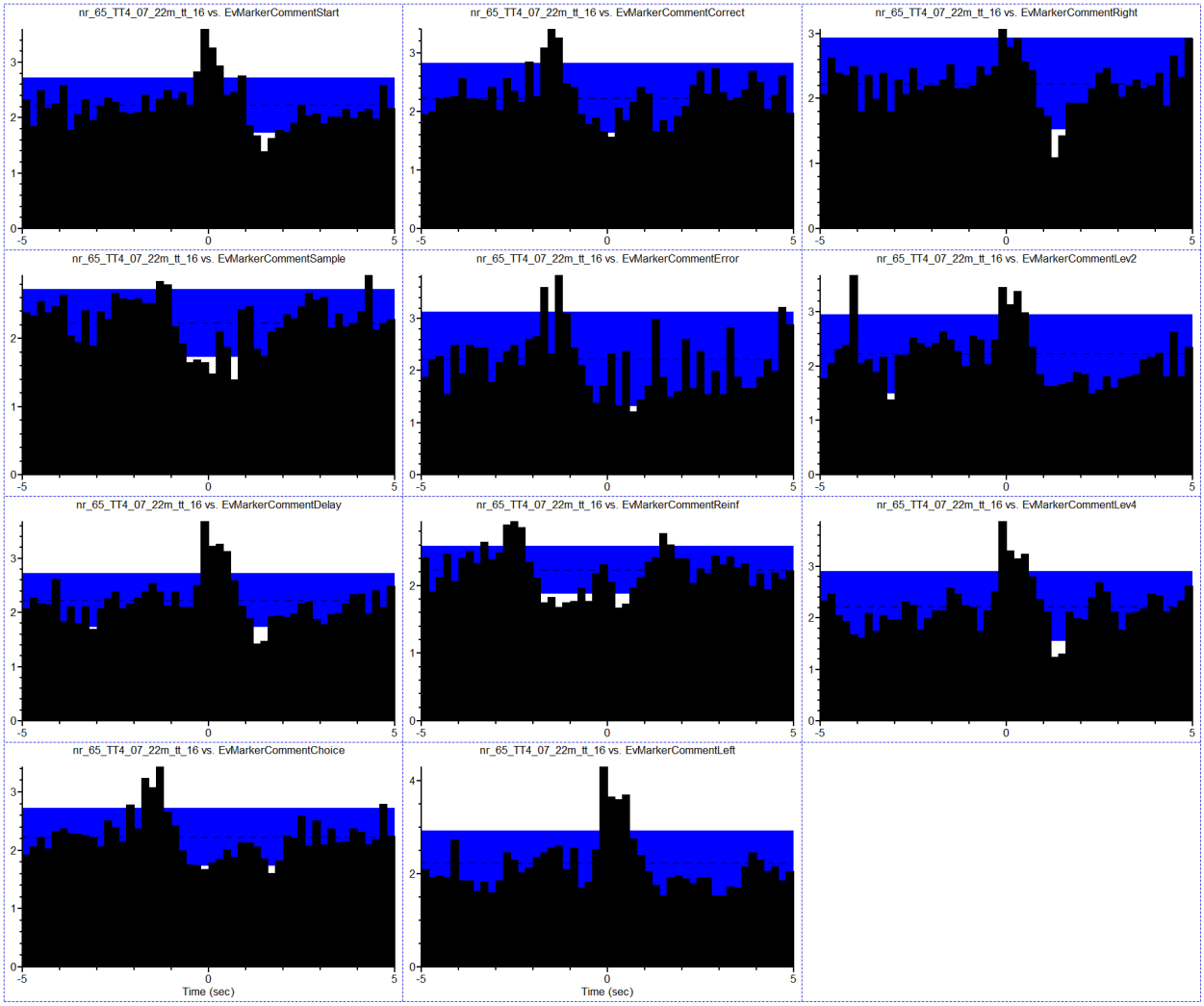


Figure 31: Base lever cell peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

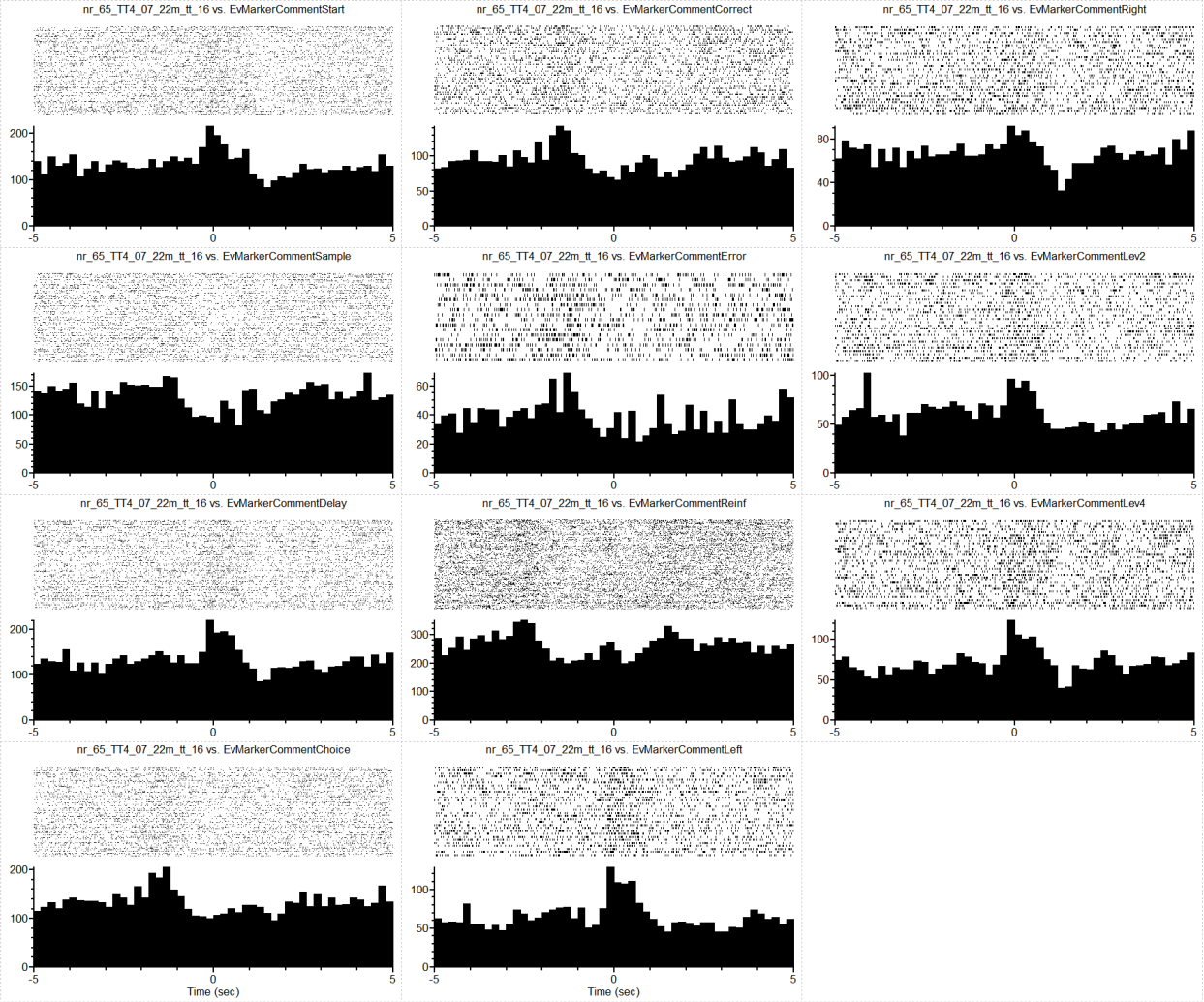


Figure 32: Base lever cell video tracking map

Base levers are located in the bottom left and top right. The highest rate of firing can be seen to the lower left lever (lever 1).

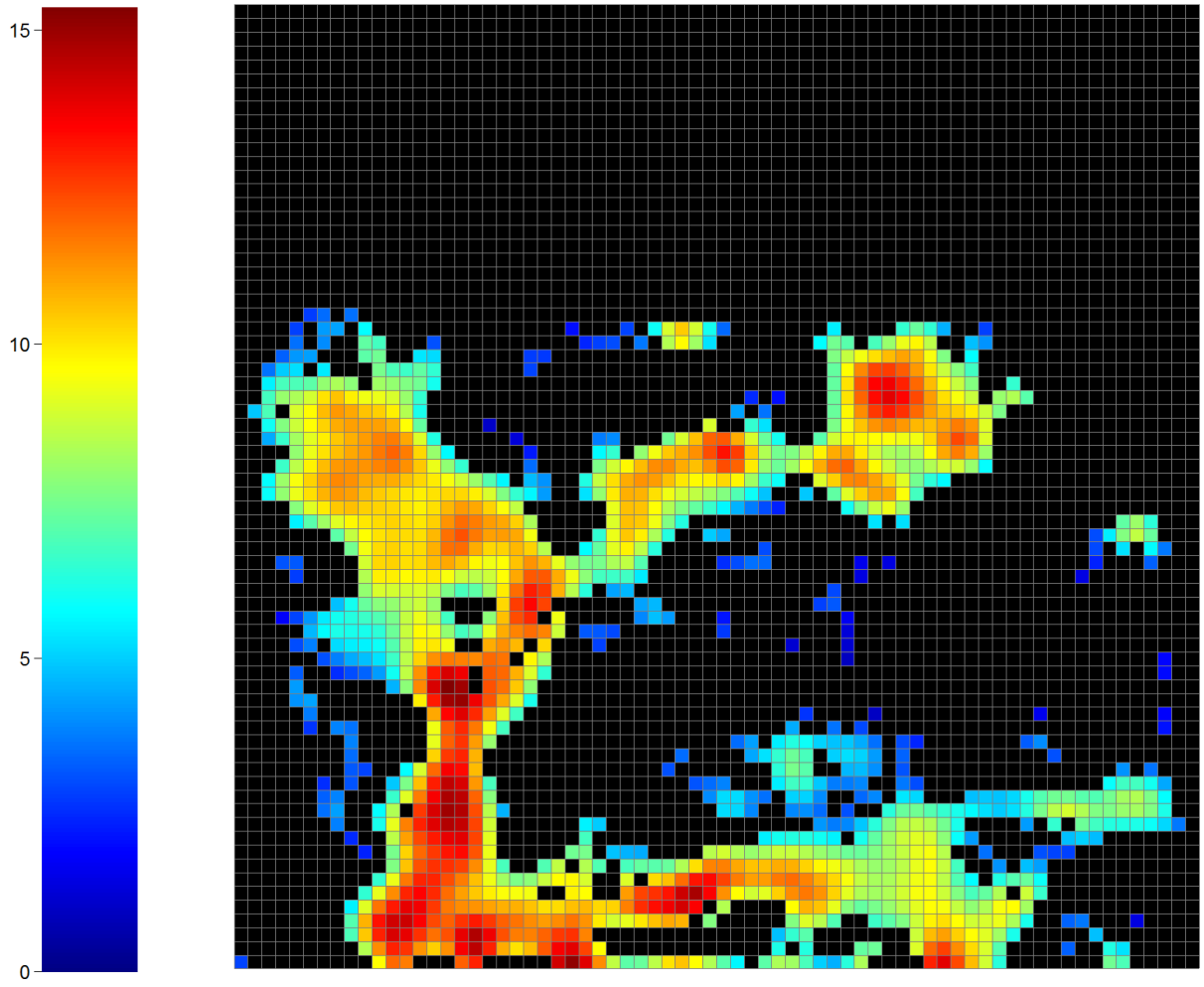


Figure 33: Complex lever press cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 1, Lever 3

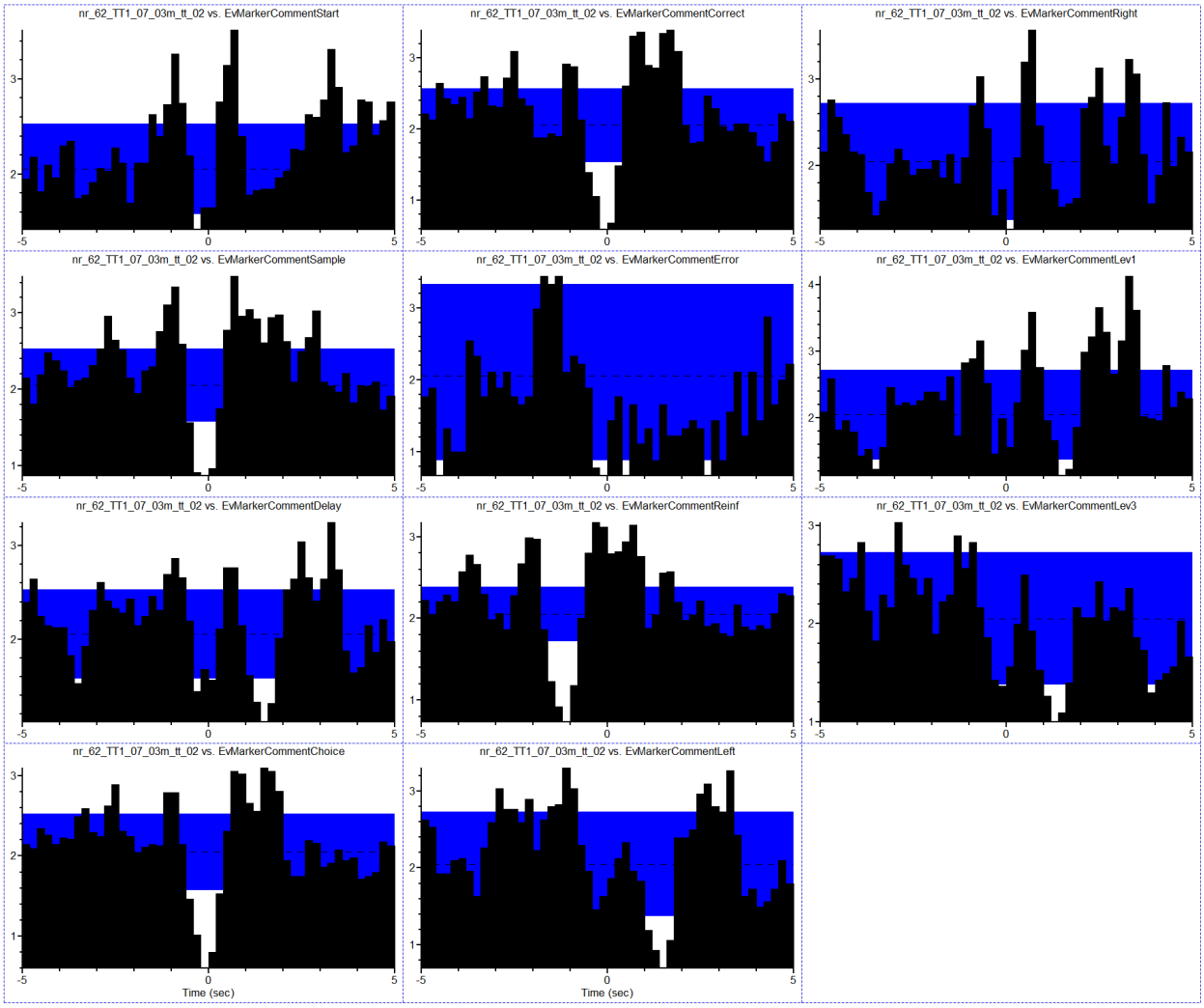


Figure 34: Complex lever press cell peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 1, Lever 3

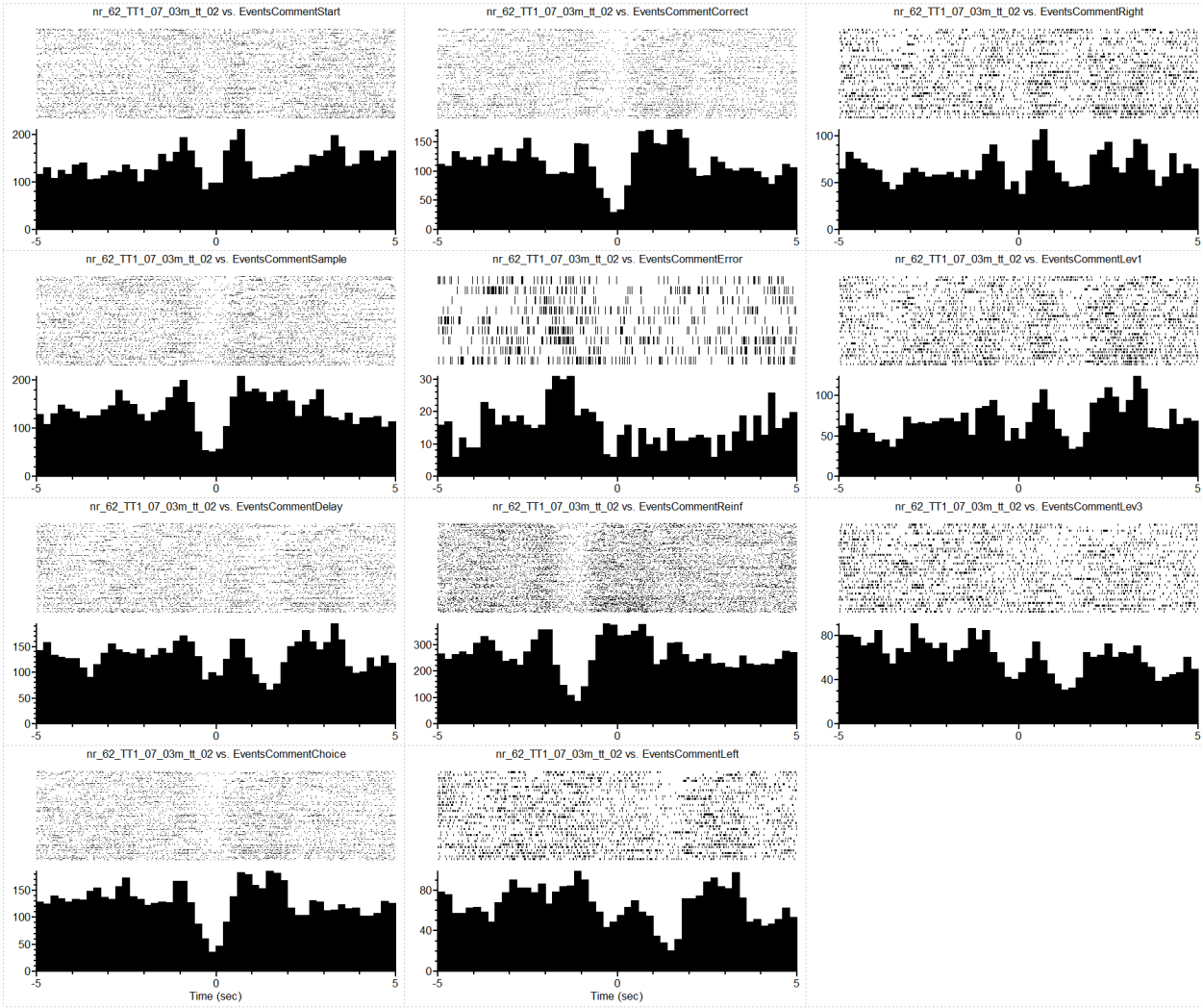


Figure 35: Preparatory cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

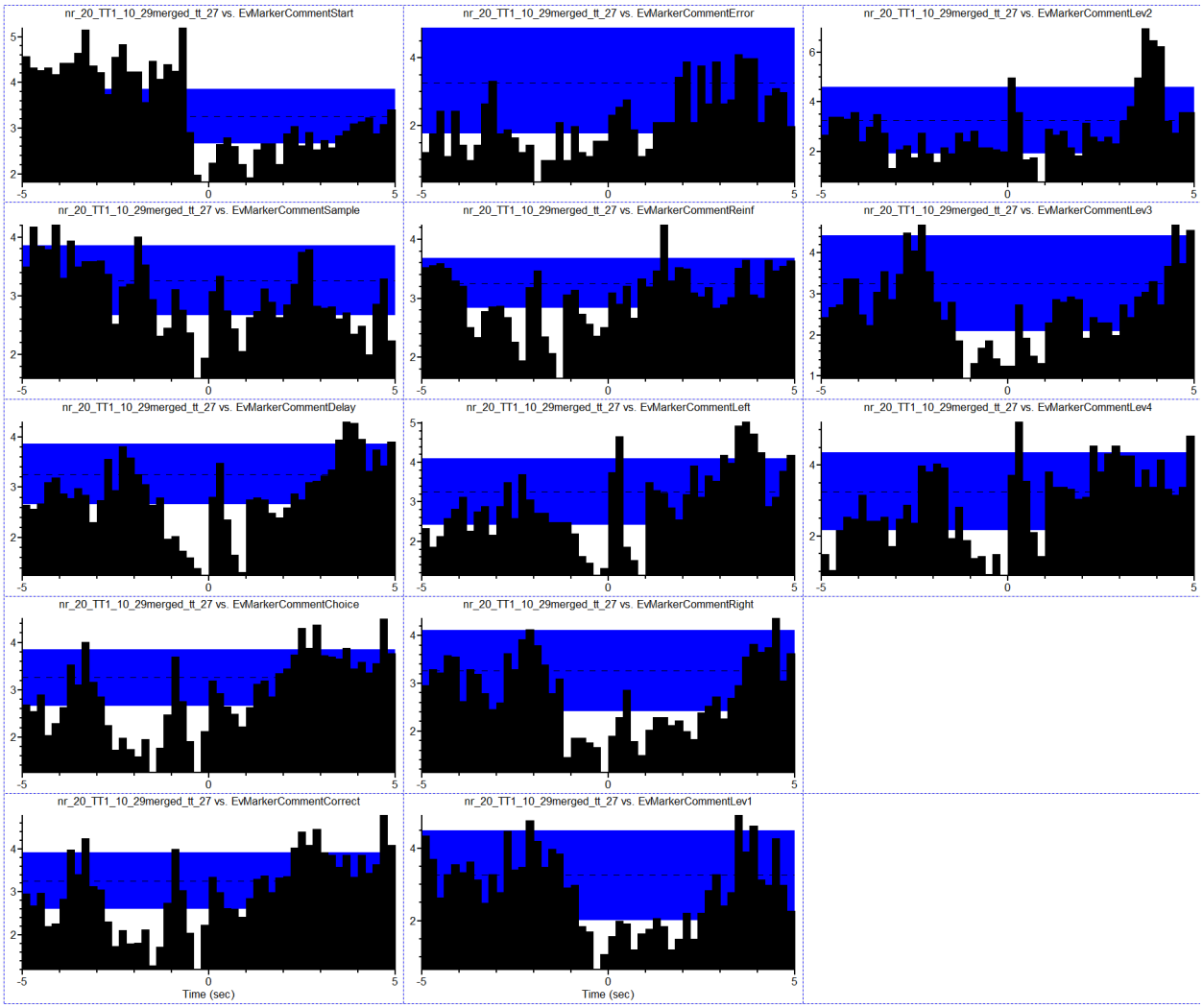


Figure 36: Preparatory cell peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

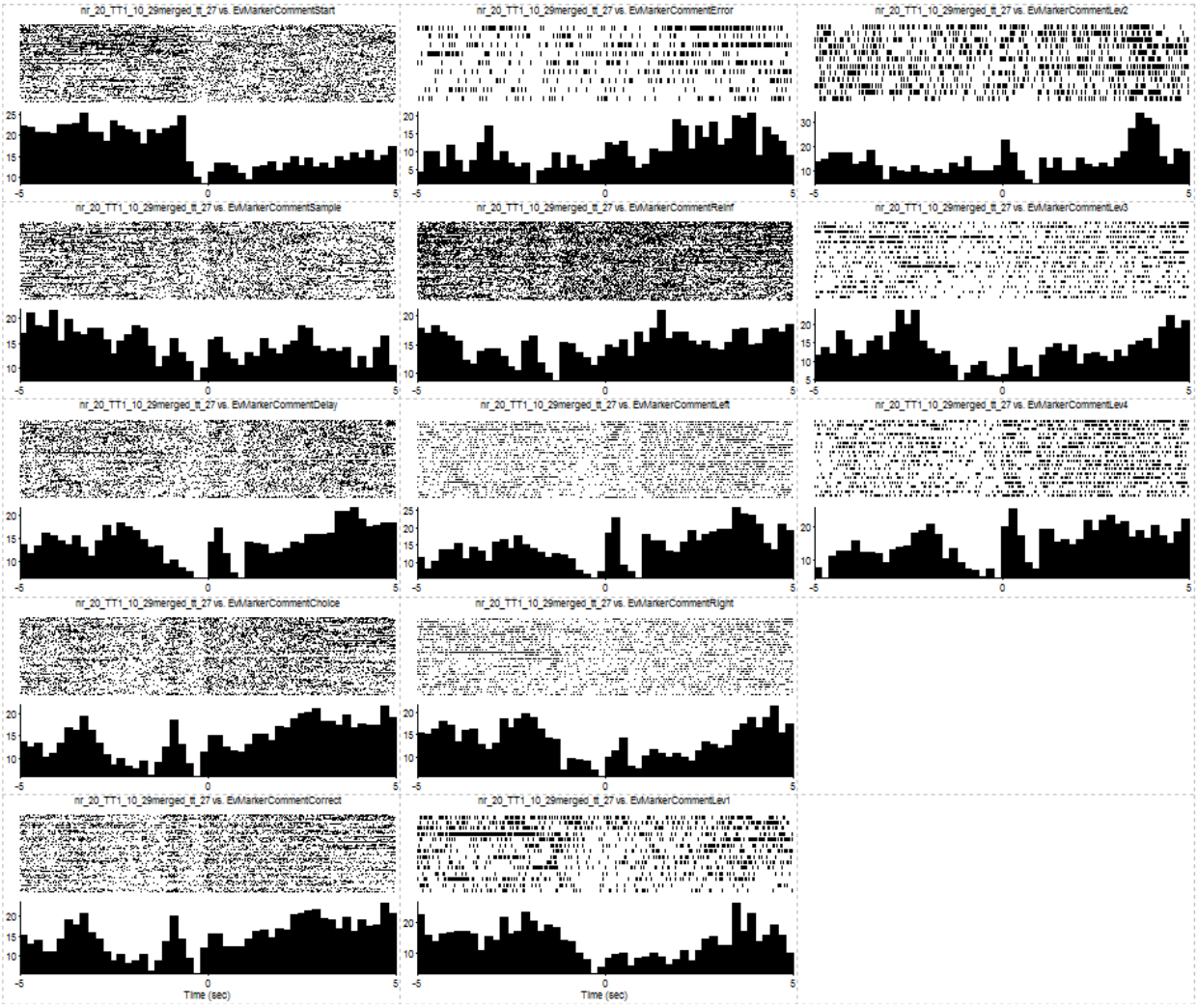


Figure 37: Delay response probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

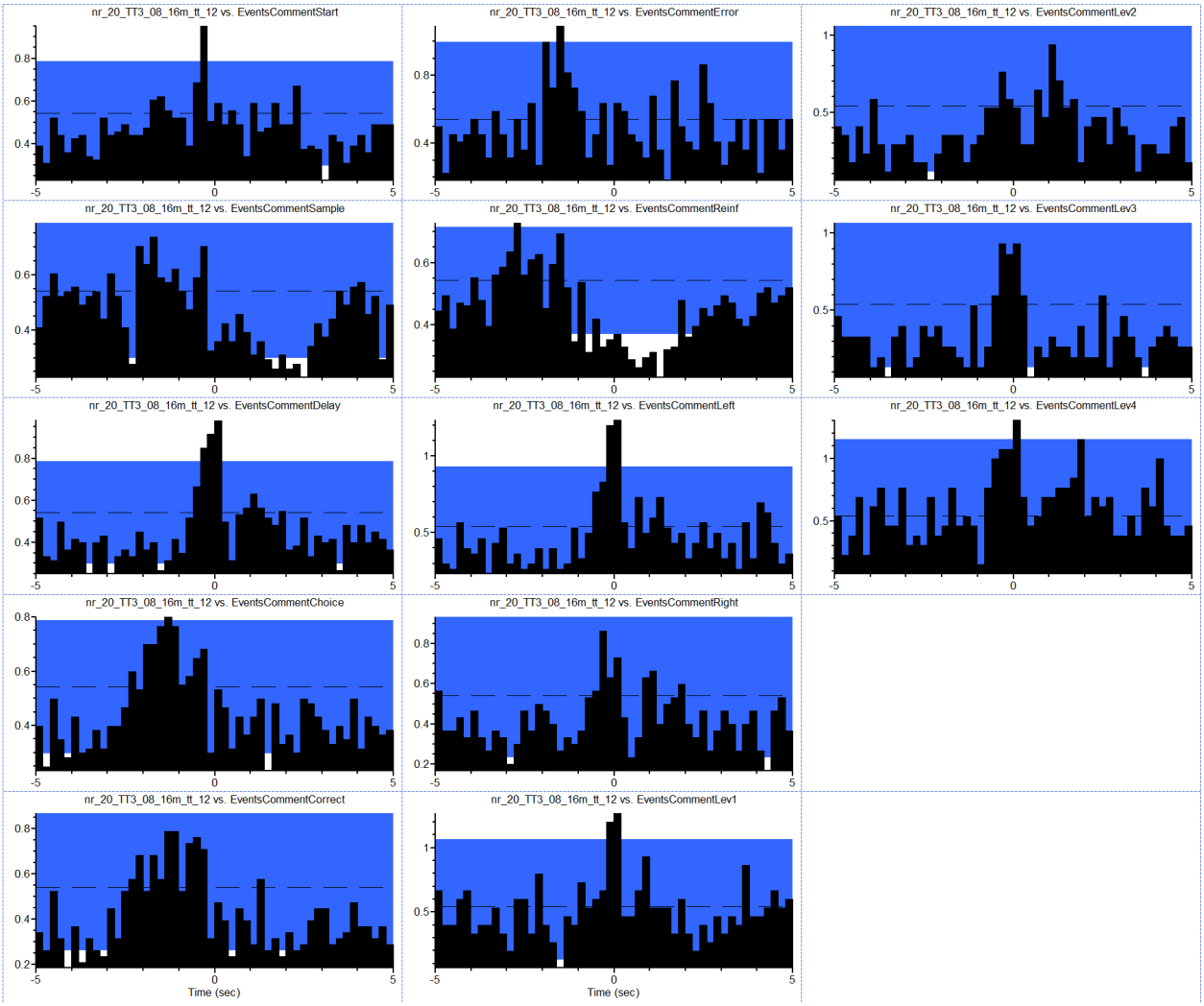


Figure 38: Delay response peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

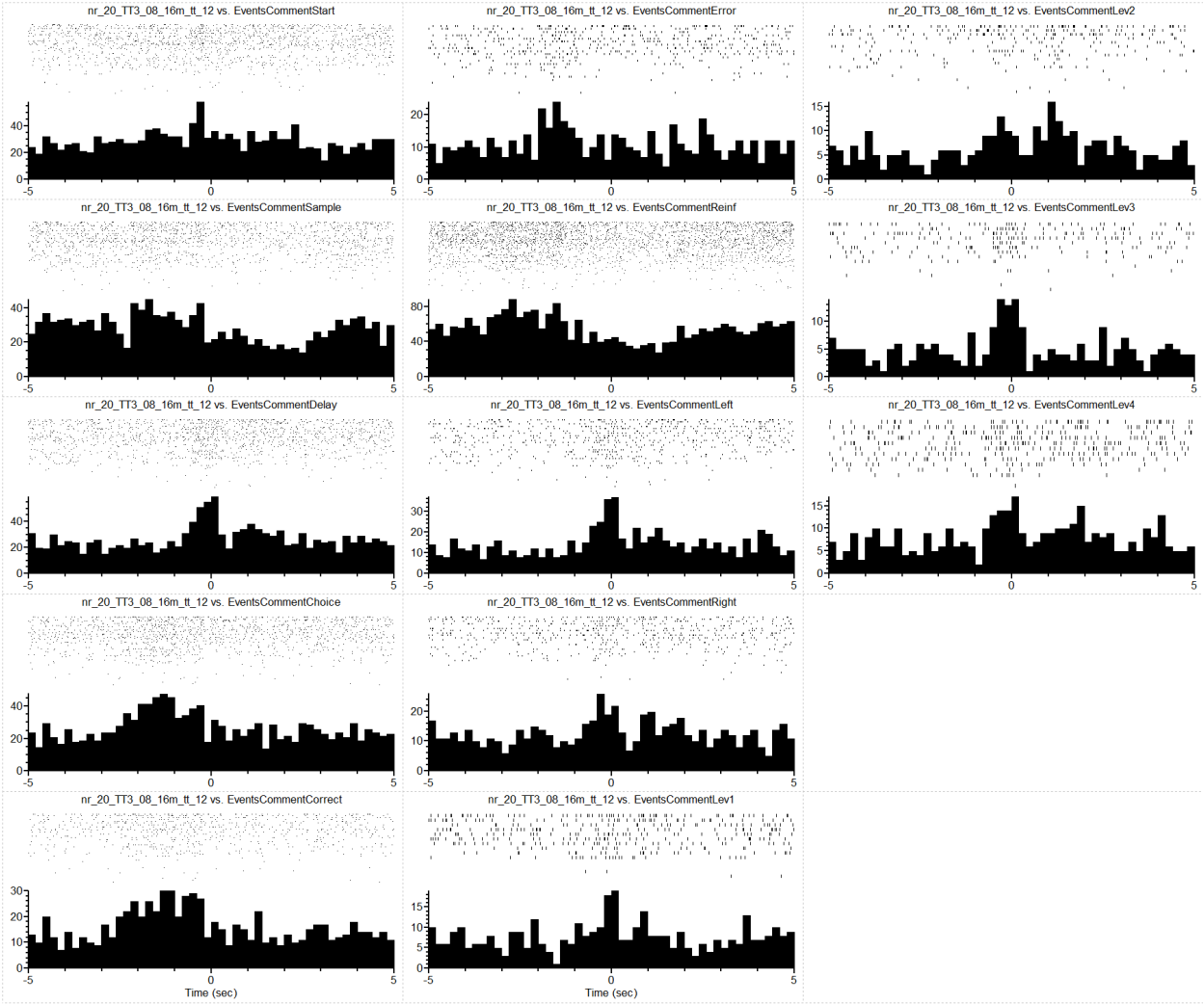


Figure 39: Location/direction specific delay related cell probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 1, Lever 3

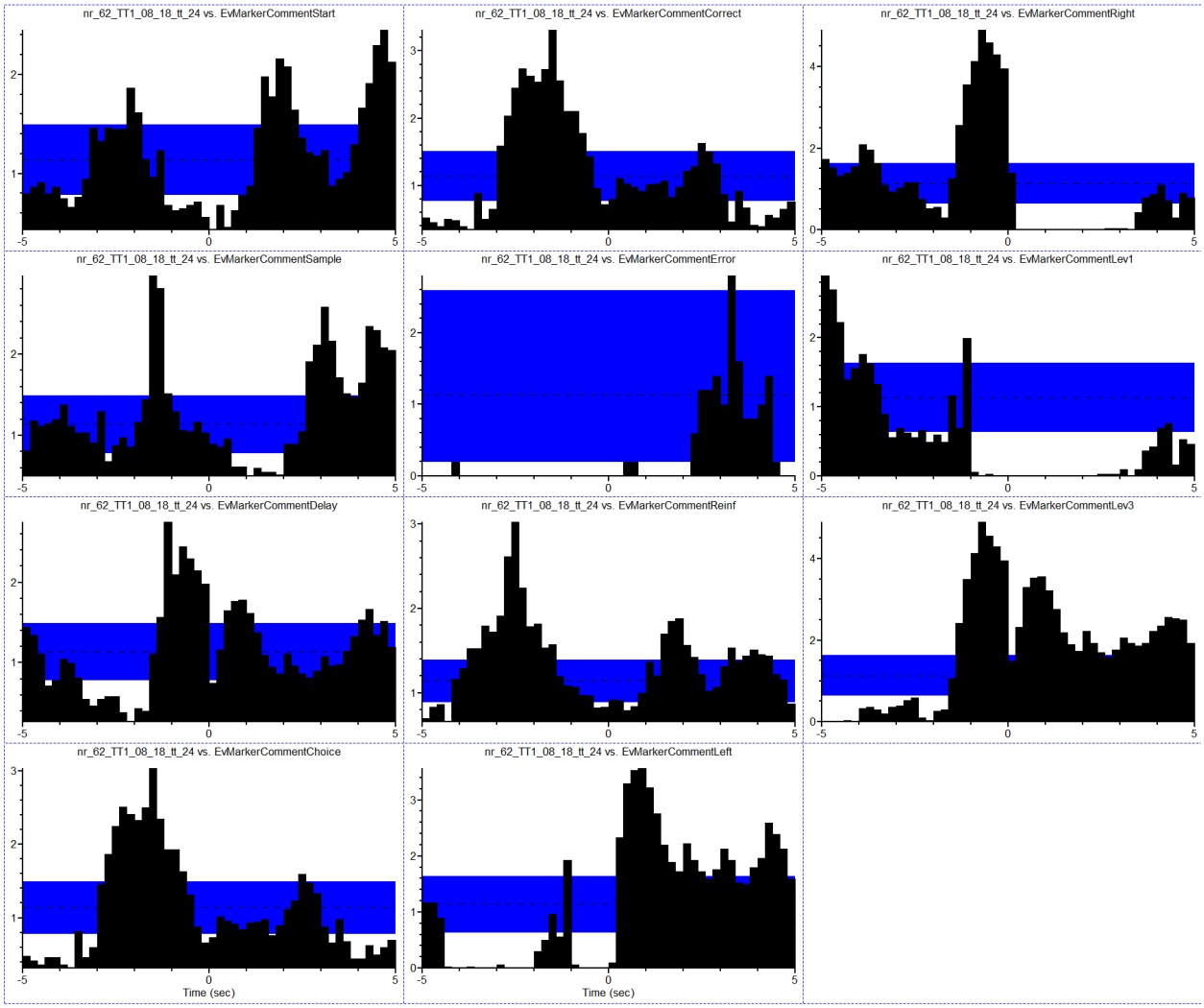


Figure 40: Location/direction specific delay related cell peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 1, Lever 3

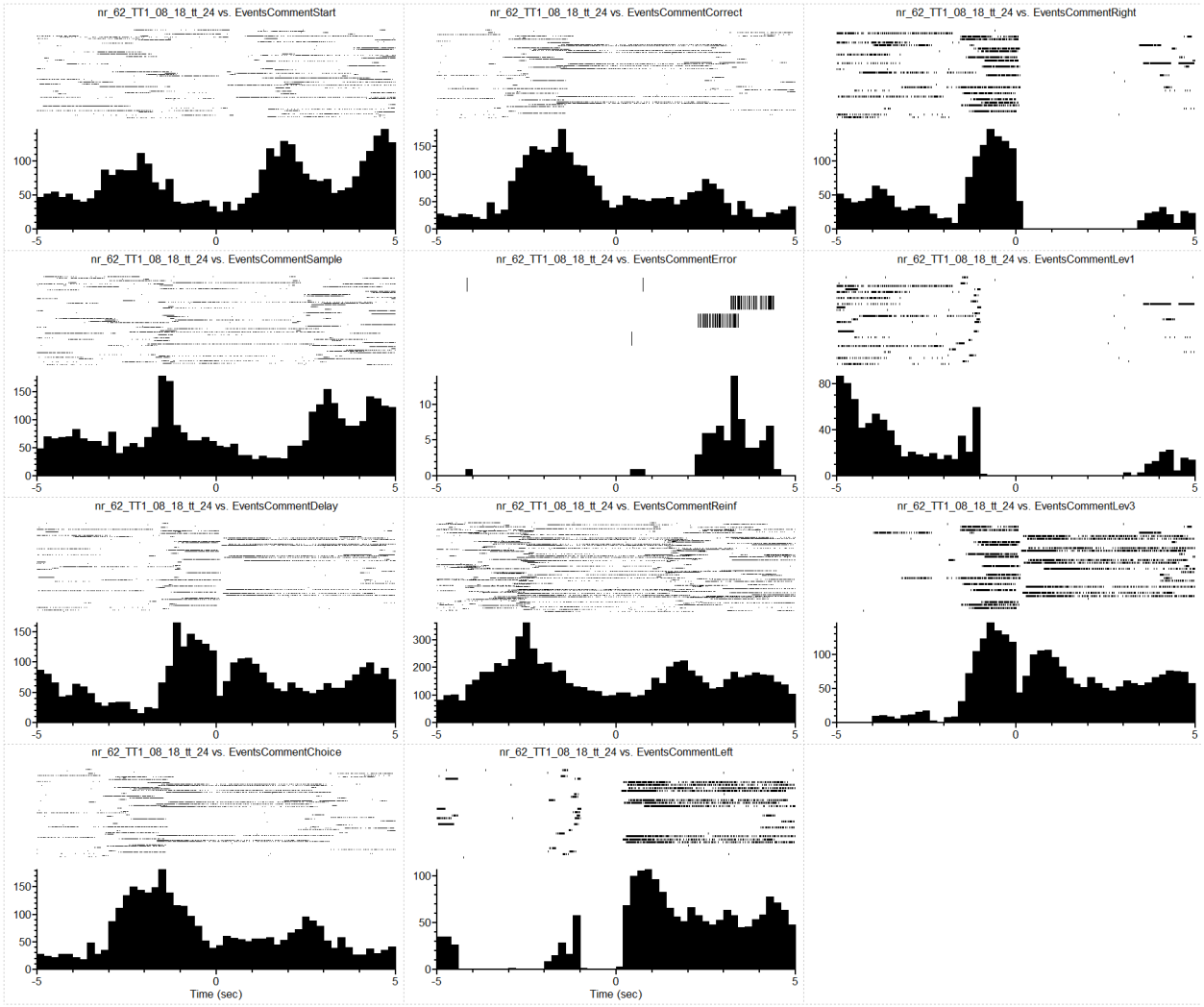


Figure 41: Reinforcement excitation probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 1, Lever 3

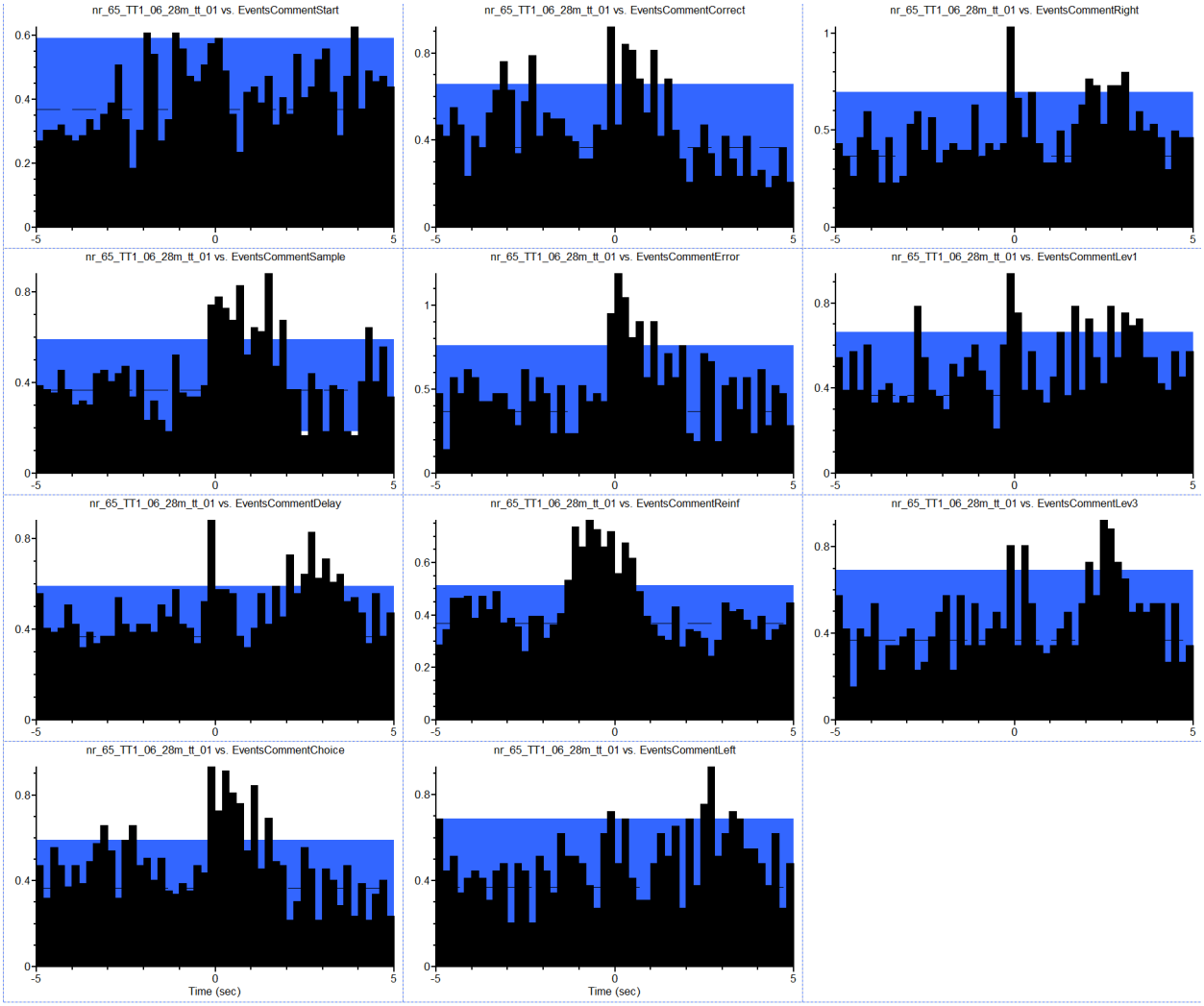


Figure 42: Reinforcement excitation peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 1, Lever 3

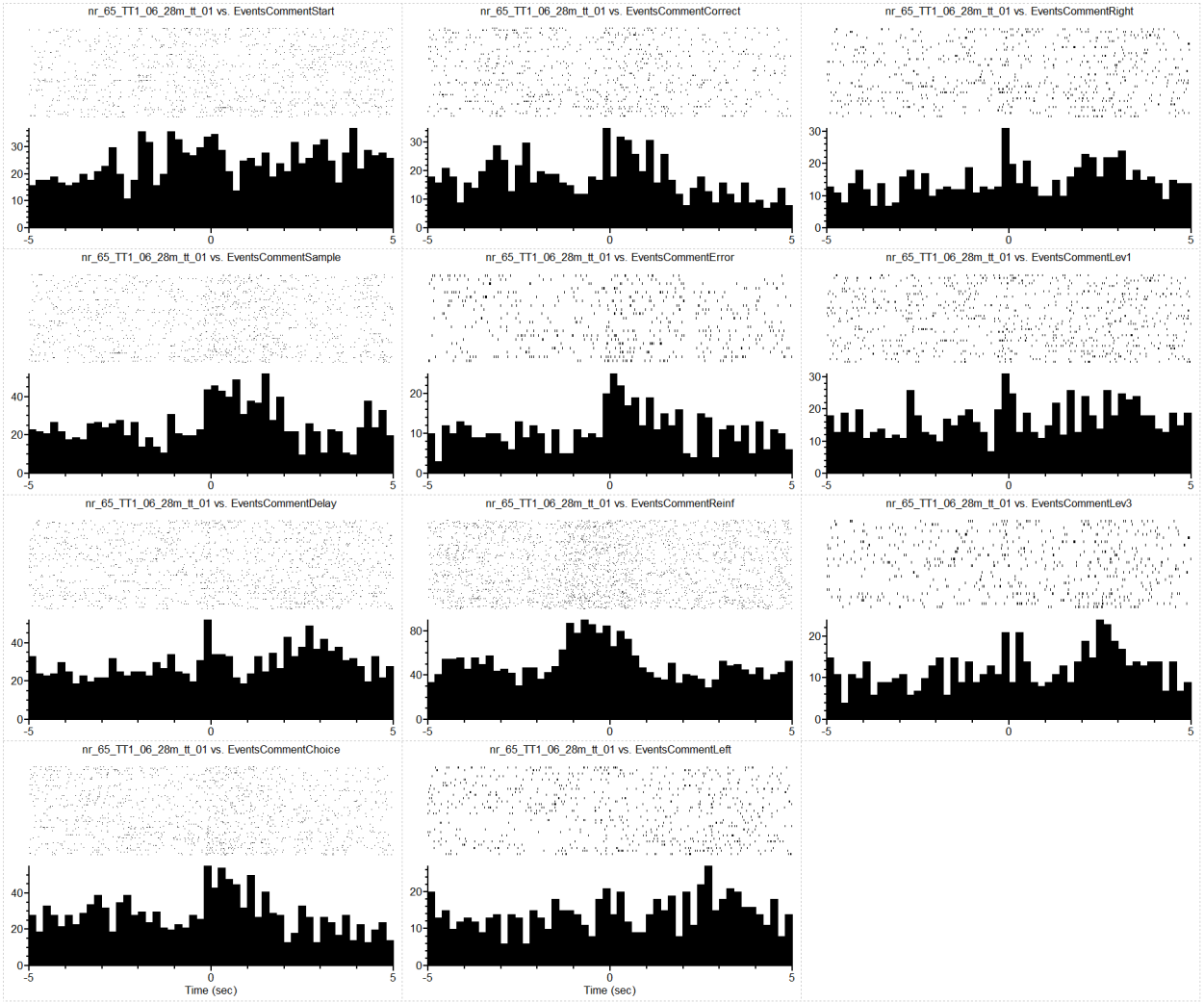


Figure 43: Right only - Reinforcement excitation probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

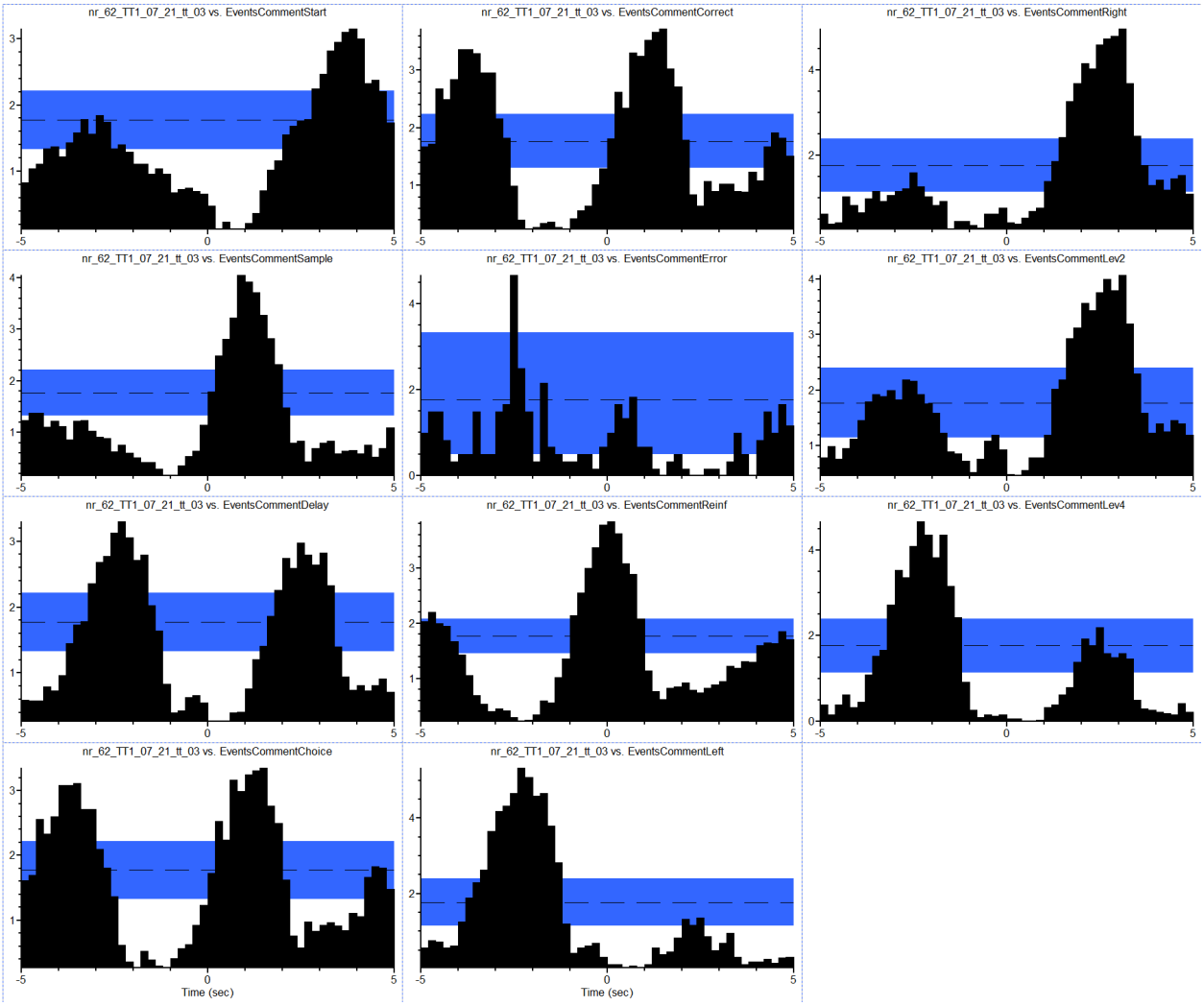


Figure 44: Right only - Reinforcement excitation peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

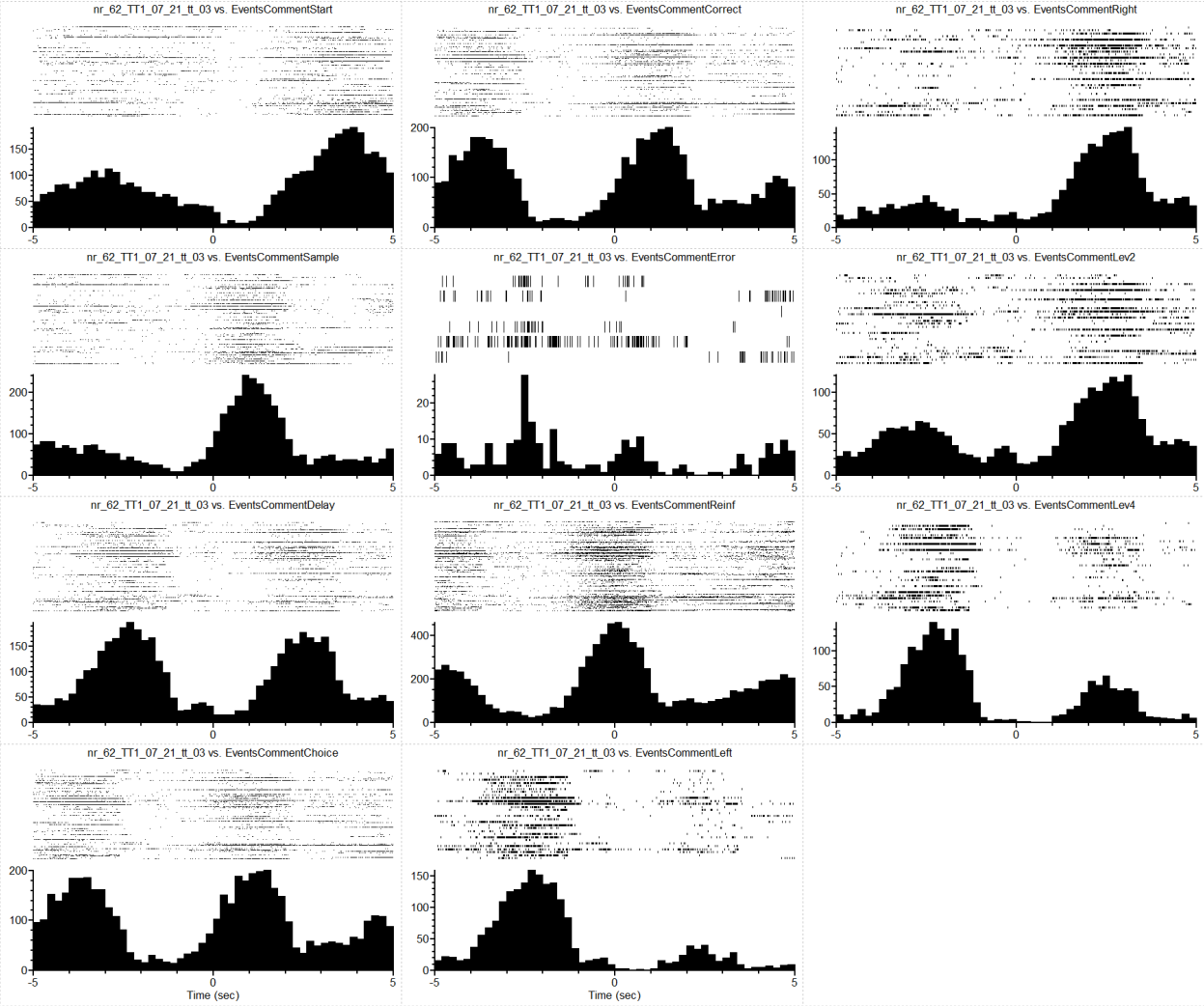


Figure 45: Reinforcement anticipation probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 1, Lever 3

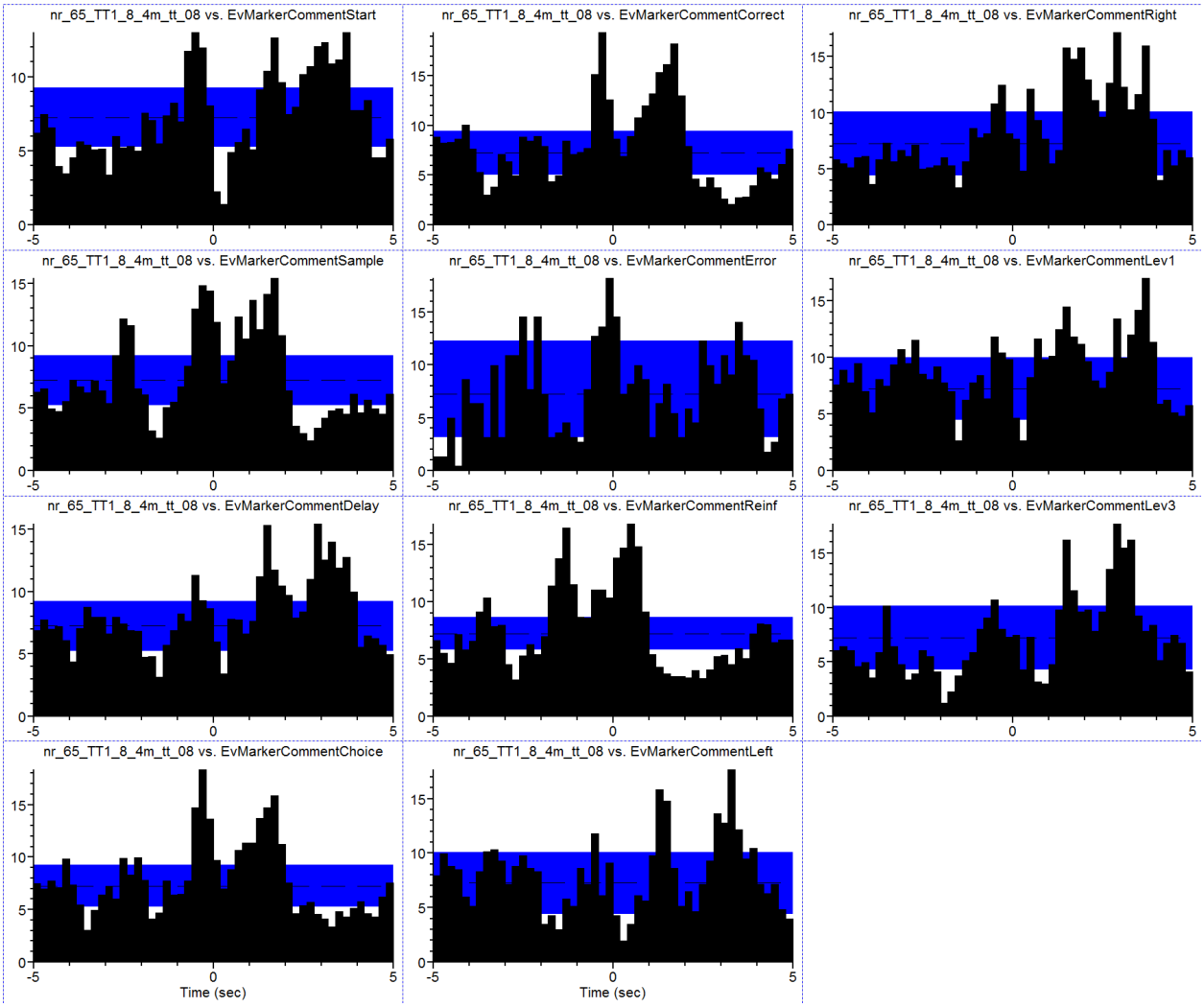


Figure 46: Reinforcement anticipation peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 1, Lever 3

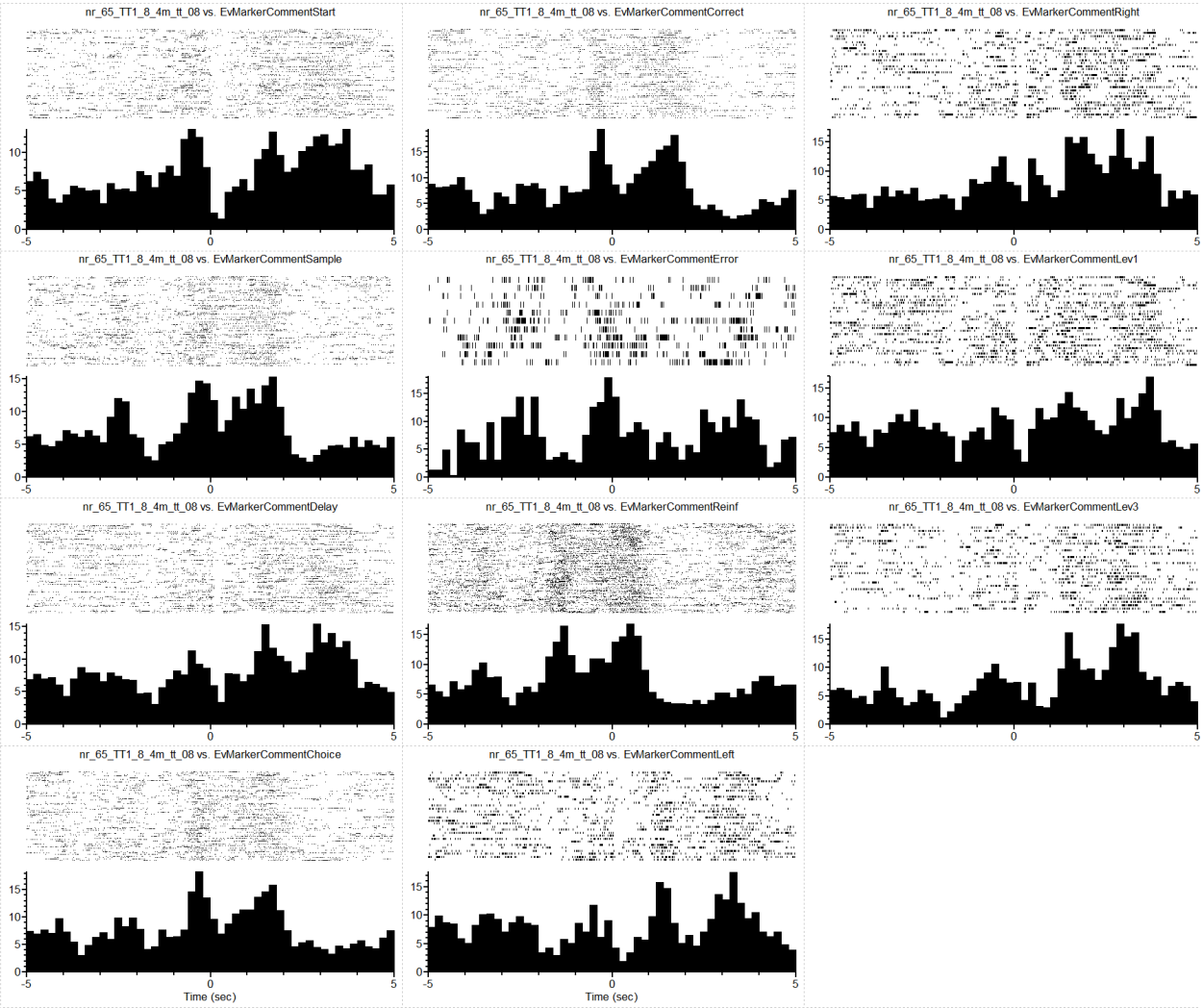


Figure 47: Error probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

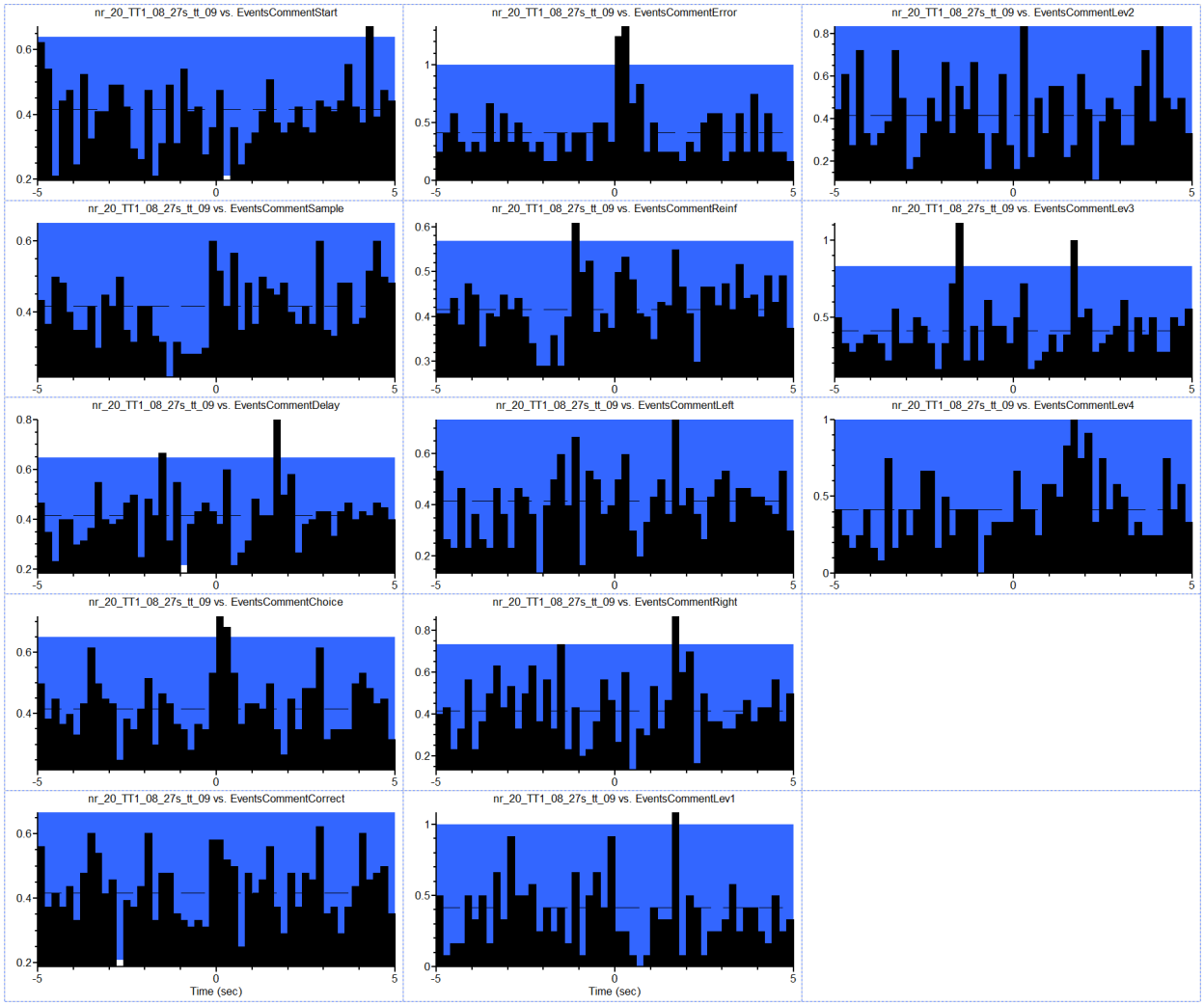


Figure 48: Error peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice, Correct. Second column: Error, Reinforcement, Left, Right, Lever 1. Third Column: Lever 2, Lever 3, Lever 4

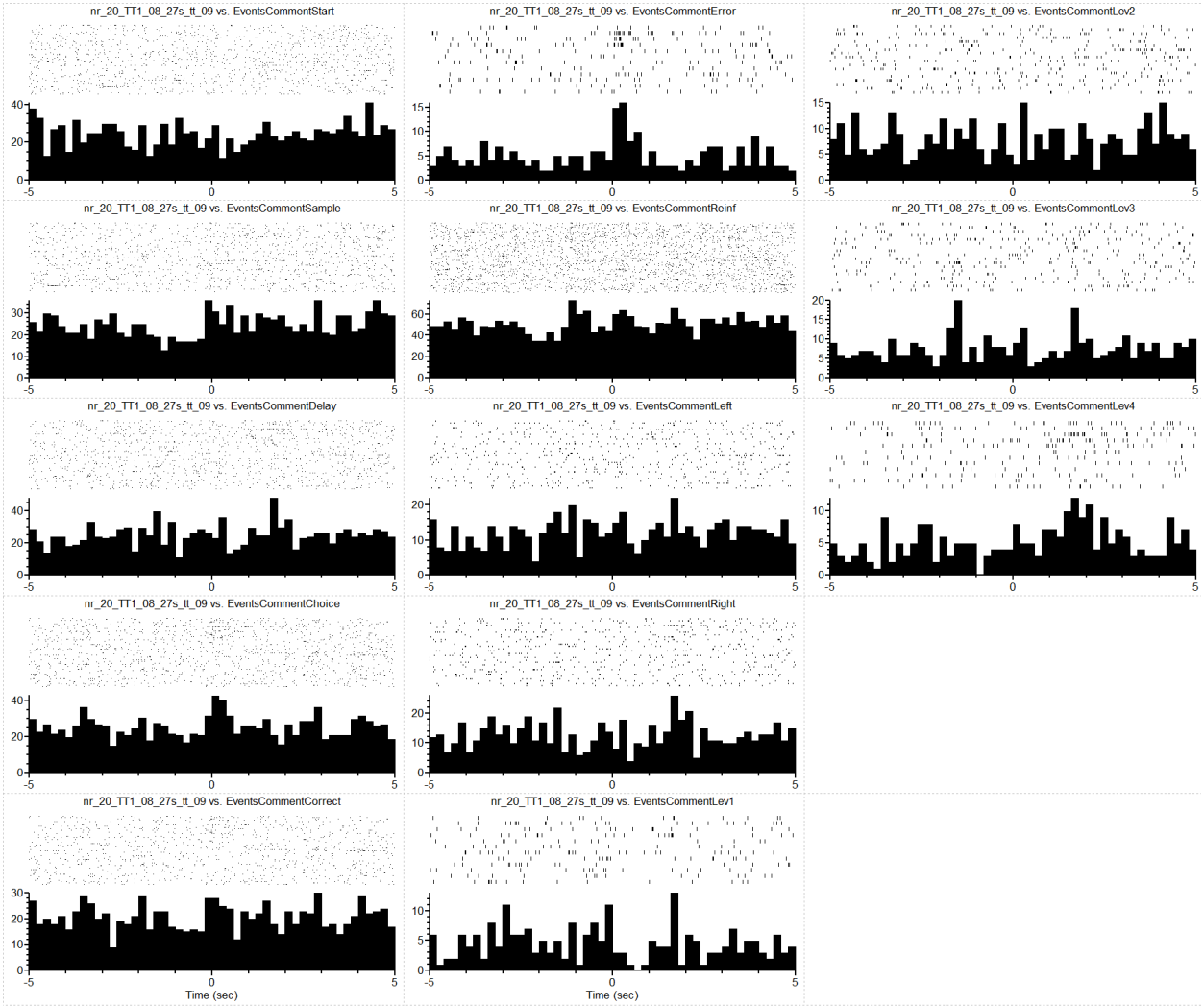


Figure 49: Action/Outcome probability histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4

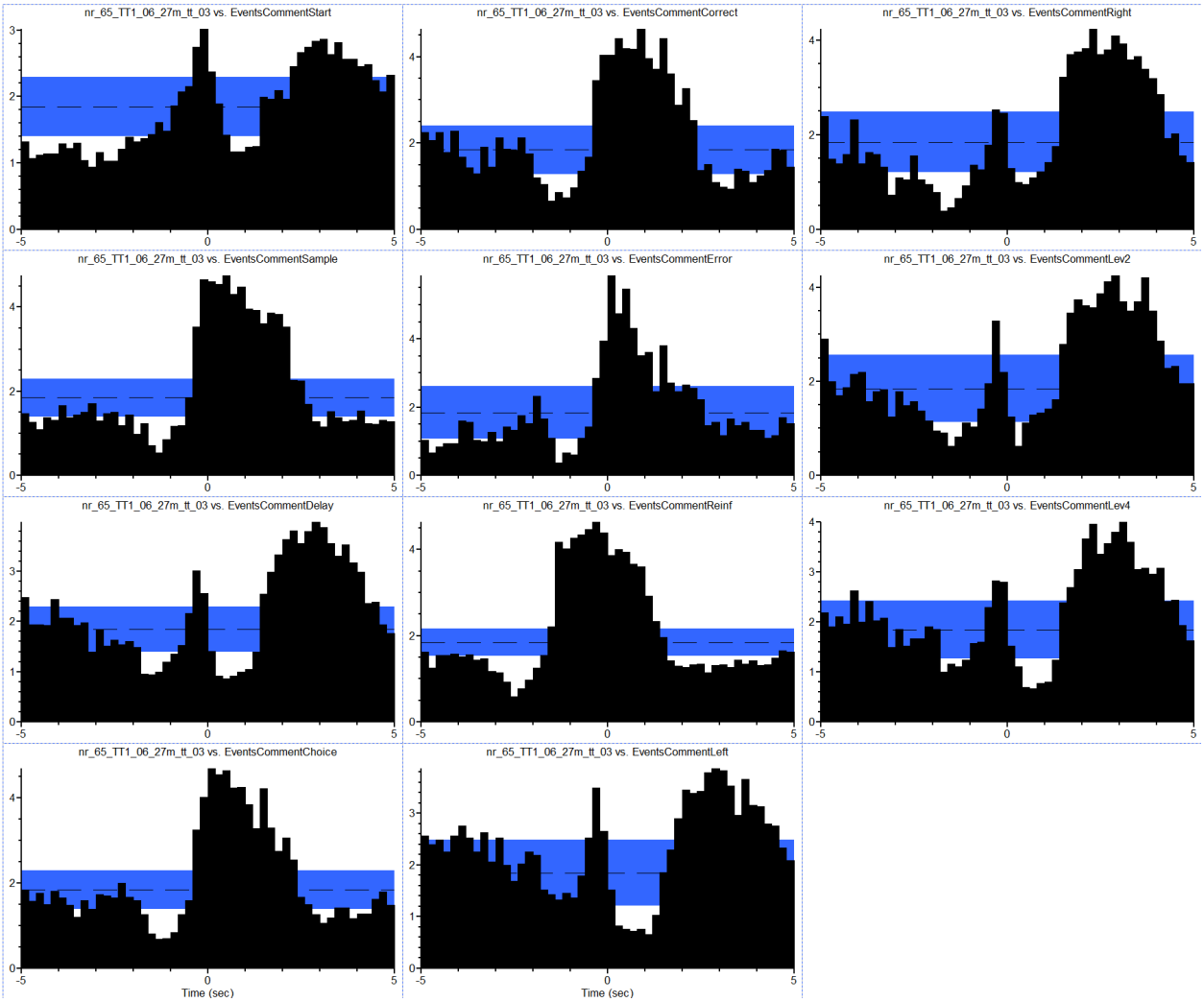
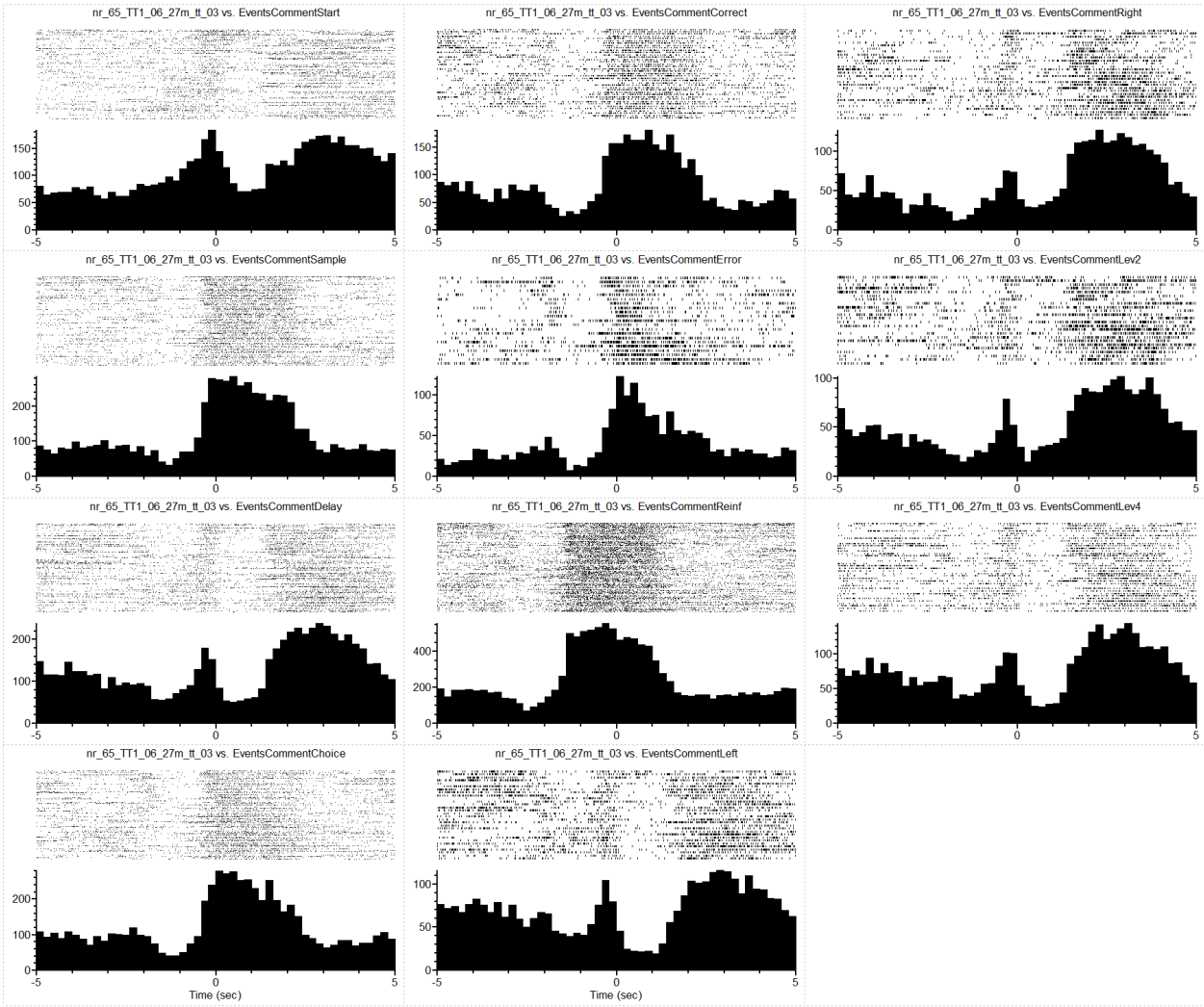


Figure 50: Action/Outcome peri-event histogram

Order of events first column from top to bottom: Start, Sample, Delay, Choice. Second column: Correct, Error, Reinforcement, Left. Third Column: Right, Lever 2, Lever 4



APPENDIX C

SLS 8/26/13

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

23-Aug-2013

Mair, Robert G
Psychology, Conant Hall
Durham, NH 03824

IACUC #: 110901
Project: Characterizing the Activity of Neurons in Thalamus During a Spatial Memory Task
Category: D
Next Review Date: 21-Sep-2014

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved your request for a time extension for this protocol with the following comment(s):

MQ 8/29/13
Lydia Manzo needs to receive occupational health program approval to handle vertebrate animal prior to doing so.

Approval is granted until the "Next Review Date" indicated above. You will be asked to submit a report with regard to the involvement of animals in this study before that date. If your study is still active, you may apply for extension of IACUC approval through this office.

The appropriate use and care of animals in your study is an ongoing process for which you hold primary responsibility. Changes in your protocol must be submitted to the IACUC for review and approval prior to their implementation.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,
Jill A. McGaughy, Ph.D.
Chair

cc: File